

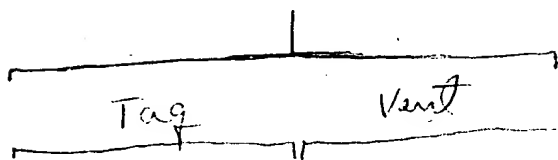
DU.GEL

- 01/19/95 - 09:20 pm

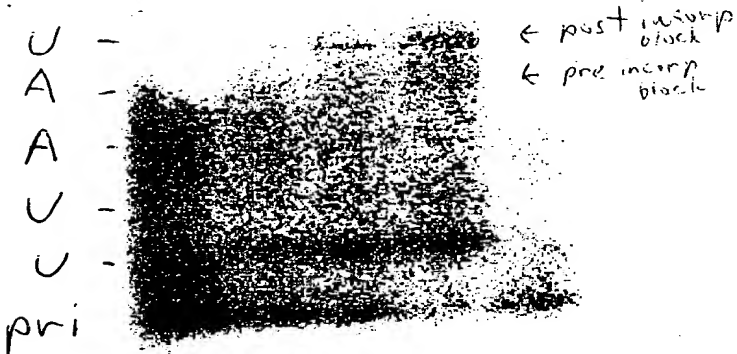
2.00x Counts

29.99  200.0

- JATP_{mut} (T) primer



min 0 2 5 10 0 2 5 10



← primer degraded

Witnessed & Undersigned by		Date	Invented by	Date	T P
			Recorded by		

Project No. _____

Book No. _____

TITLE _____

Miniprep for Ayoab
 PLAC PCR

114

From Page N _____

miniprep #

1-20

21-40

41-60

61-80

81-100

101-120

121

Tag

+

+

+

+

+

+

+

Tag + Deep. Val

+

+

+

W/N

0

.05

0.1

0

.05

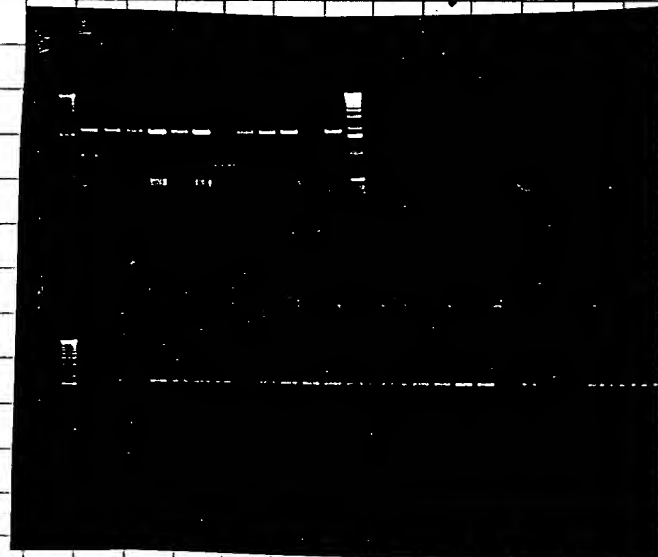
0.1

Blue colony

grow 1/10 30°C, 2 ml circle grow + 100 µg/ml Amp

miniprep same as p41, 4 using 1 ml cells

digest as per P 93 AIF IV Act II, Eco RI 5 µl miniprep
 1-40 on 40 well comb, load 10 µl
 conclude resolution not good
 enough for ~500 bp range
 1-40 on 30 tooth comb, load
 need more DNA in digest and load 2



10

20

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Deena Polansky

Date

2/16/95

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Record d by

Dat

127-55
 3075

T Pag N

Repeat digest of P114 for fidelity
assay: use 10 μ l miniprep

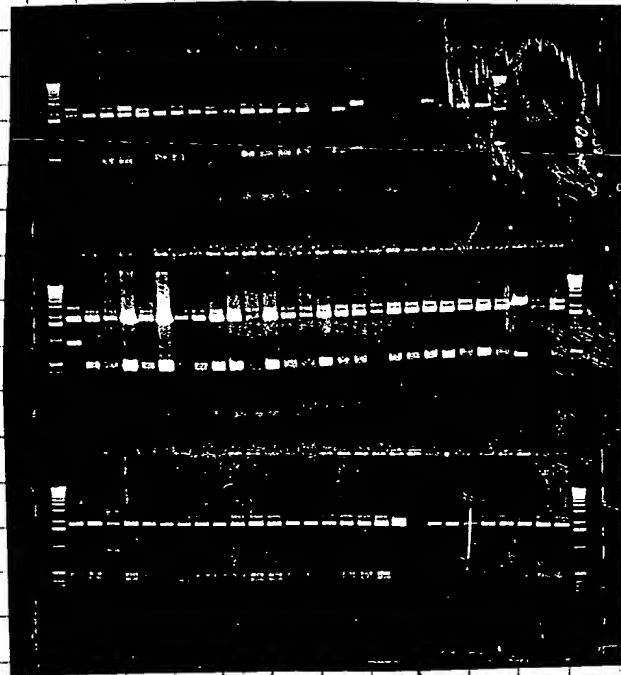
ag N

digest 2h 37°C load 20 μ l

NEB buffer	2 μ l	✓
1/4 Afl III	0.3	
1/4 Aat II	0.1	
1/4 Eco RI	0.5	✓
H ₂ O	7.1	✓

VP = 10 μ l

digest 10 μ l miniprep
VP = 20 μ l



miniprep

5-7-8

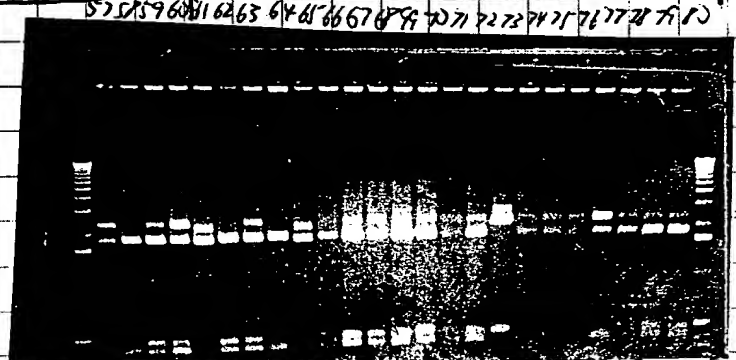
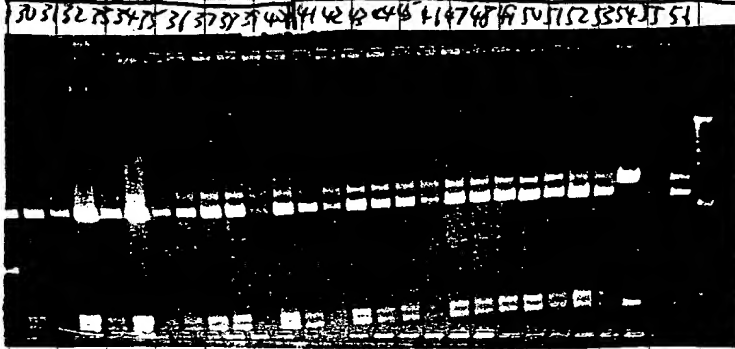
29-56

1-28

0.5 mm Mn

1 0.1 mm Mn

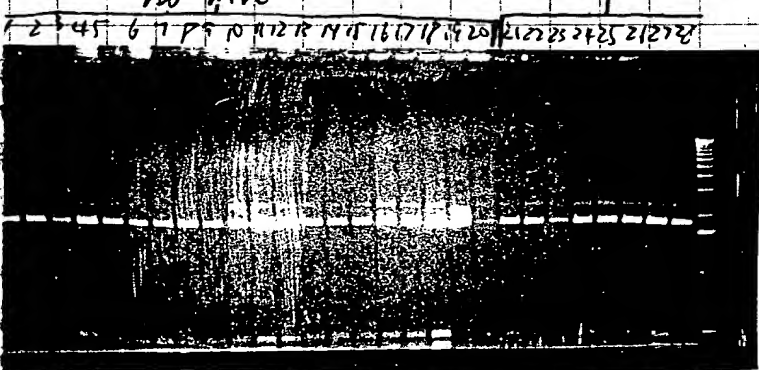
no Mn, + Vent



160
2
11
47
47

no Mn

0.5 mm Mn



Del ↑ ? ↑ ?

full length
for #13
rel P123

To Page No. _____

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Date

1-30-95

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Book No. _____

Results P 115

116

From Page No. _____

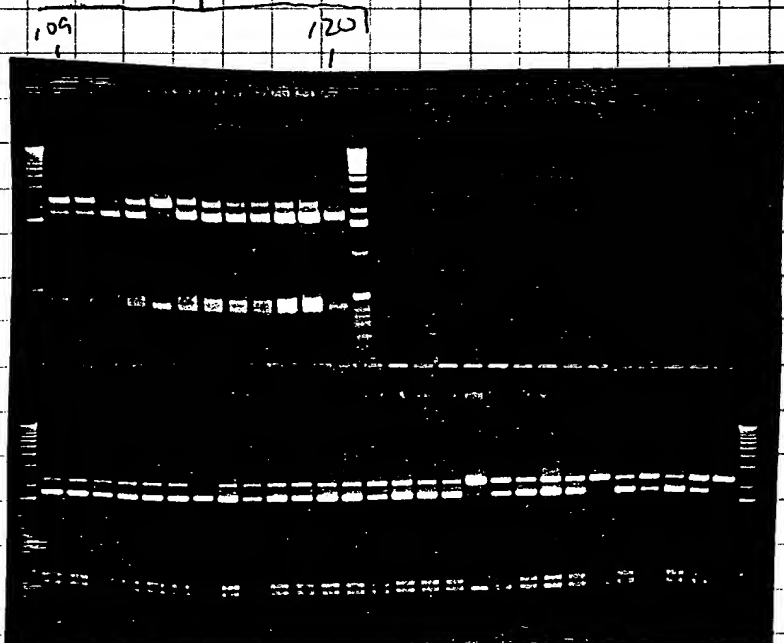
	full length	+ 410 bp (no Ant II)	+ 465 bp (no Ant III)	(?) deletion	gDimer	Res
#1-20, Tag 0 Mn	17 19	(Q32 P 123 SST)		1	X	✓
21-40, Tag 0.05 mM Mn	18 20				X	✓
41-60, Tag 0.1 mM Mn	18	2				
61-80, Tag 0 Mn + D Vent	12 17	X	1	2		4
81-100, Tag 0.05 mM Mn + D Vent	18	2				
101-120, Tag 0.1 mM Mn + D. Vent	16	4				

See new Table on P 124 after Dr. I and SST cuts

- ⊗ 900mer lacks RI site in MCS
- ⊕ no result, i.e. not enough DNA to be sure about cut.

confirmed deletions
miniprep #19, 61, 65

0.1 mM Mn, Tag + Deep Vent



only 410 or 465 removed
so it's 2.2 bp
1.8 bp
has 410 and 465 removed

miniprep #

0.05 mM Mn Tag + Deep Vent 90 100 108
0.1 mM Mn Tag + Deep Vent

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		Recorded by	

Page N

Still Needed 8

cut with Ora I to see if full length lac Z is present (assuming either Afl III or Aat II recognition region had a point mutation generator). There are the "410" and "465" bp

miniprep # 54, 58, 64, 73, 87, 98, 103, 108, 113, 120

plus Aat II , Afl III

cut with Sst I to see if R1 site in MCS was a point mutation (or very small deletion (all on P(07 at bottom) resulting in the "90mers")

miniprep # 3, 29

Recut with $\text{17 } \mu\text{l}$ miniprep and load $30 \mu\text{l}$?

^{25 μl reaction}
trying to resolve the "No results"

miniprep # 20, 39, 71, 74, 75, 76

To Page No. _____

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Erica Boland

Date

2/16/95

Invented by

Recorded by

Date

1-31-95

From Page No. _____

SAP	CPM1	TIME
A1	1 4976.00	0.50
	2 5216.00	0.50
	3 4500.00	0.50
	4 16920.00	0.50
A6	5 17020.00	0.50
	6 16156.00	0.50
	7 3926.00	0.50
A7	8 3822.00	0.50
	9 4054.00	0.50
	10 15974.00	0.50
A2	11 16520.00	0.50
	12 15478.00	0.50
	13 4684.00	0.50
A3	14 4752.00	0.50
	15 4606.00	0.50
	16 17622.00	0.50
A8	17 16806.00	0.50
	18 17742.00	0.50
	19 4186.00	0.50
CTI	20 3966.00	0.50
	21 3986.00	0.50
	22 14842.00	0.50
LTI	23 14704.00	0.50
	24 15620.00	0.50
	25 4458.00	0.50
L	26 4644.00	0.50
	27 3970.00	0.50
	28 16730.00	0.50
L	29 16914.00	0.50
	30 15684.00	0.50
	31 4864.00	0.50
L3	32 5020.00	0.50
	33 4538.00	0.50
	34 15236.00	0.50
L18	35 17922.00	0.50
	36 17898.00	0.50
	37 12.00	0.50
Aqueous	38 16.00	0.50
blank	39 16.00	0.50

delivered 10 μ l with p10 (wiped tip)
 rinse 3x into 4 ml aqueousol

each dilution had 3 μ l of 1 μ C/ml 3H TTP

To Page 1

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Date

Invented by

Date

Recorded by

Deena Boland

2/16/95

RJP

1/25/95

New rTag dilutions

Proj ct N . _____

Bo k No. _____

119

ag N . _____

#

EKB T1

77.4

18.6 μ l

323 units/ μ l (P91)

Tag dilution buffer

4922.6 μ l

1981.4 μ l

$V_f = 5$ ml

(5 units/ μ l)

$V_f = 2$ ml

(3 units/ μ l)

both are labelled "1-31-95 rTag"

To Pag No. _____

ss d & Und rstood by m ,

Date

Inv nted by

Date

Deena Bolap

2/16/95

R cord d by

1-31-95

Project No. _____

Book No. _____

TITLE

Accuracy of delivering 1 μ l
with P2 pipetman for Tag storage

120

From Page No. _____

add 1 μ l, 10 times to a weigh boat with
a drop of H₂O in it so tips can be rinsed
several times. Use storage buffer at 0°C (on ice)

add H₂O Total 0.0000

1 μ l
2

3

4

5

6

7

8

9

10

0.0119 $(\frac{94}{103}) = 0.011$

note 10 μ l SB = 0.01
.0094

So ~ 1.1 μ l was added instead of the 1 μ l intended

conclude 2 μ l is better to add for units

Stock	for Tag unit assay	used	CP in unit assay	for 6607 Rxs
3 μ l	0.5 M TAPS pH 9.3	150 μ l	25 mM \rightarrow	✓ X 206
120 μ l	1 M MgCl ₂	6 μ l	2 mM ✓	0.1 M DTT ✓ X 3
1 μ l	3 M KCl	50 μ l	50 mM ✓	10 mM DTPA ✓ X 6
				12 mM Mg/ul Salamm ix 7340
				Tarbo Chloral/UNA ✓ X 12
				H ₂ O ✓ X 293
				CP = 3

use 230 μ l / 2 μ l Tag unit assay mix

Witnessed & Understood by me,

DeeAnna Polamp

Date

2/16/95

Invented by

Recorded by

Date

2-1-95

Page No.

Proj ct No. _____
B ok No. _____

123

Continued from P 111
SSTI and DrdI cuts of mutants

age N	mutant	control	control
	(1) 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21		
up #	30 54 58 64 73 87 92 103 108 113 120 30	3 29 30 20 39 71 74 75 76 31	
buffer 4	2 2 2 2 2 2 2 2 2 2 2 2	2 2 2 2 2 2 2 2 2 2 2 2	
I	1 1 1 1 1 1 1 1 1 1 1 1	1 1 1 1 1 1 1 1 1 1 1 1	
+			
+			
- R1			
		0.3 → 0.45 →	
		0.1 → 0.15 →	
		0.5 →	
		0.75 →	
		2.1 →	
		20 μl	30 μl

37°C 2 hr
was only ~ 1/2 cut by 1 hr
add 0.5 μl more ~~any~~ DrdI for last 30 min
for DrdI

miniprep #	6p fragments	Results
control	2.7 kb (mut), 1.8, 0.8	full length sucrose
# 73	"	"
# 58, 54	"	"
# 87, 120	"	"
# 54, 103	"	"
# 113	"	"



1.8
miniprep 3 and 27
have full length loc based on presence of 410, 465 bp
So R1 was probably small on point mutation
all no results of R1
all full length loc based on presence of 410, 465 bp
same mode OK cut here
minic # 73
does not have full length loc is present

Read & Understood by m ,
easier a Polarp

Dat 2/16/95

Invented by [Signature]

Recorded by [Signature]

Dat 2-2-95

To Page No. _____

From Page No.____

Full length
line

percent rearrangement

maniprep #

M_n (mM)

Deep Vent

1-20

0

19

50%

21-40

05

20

A diagram showing a closed, irregular curve. A vertical line segment passes through the curve, starting from a point above it and ending at a point below it. The line segment is labeled with a small 'a' at its top end and a small 'b' at its bottom end.

41-60

0.1

18

10 6 4

61-80

0

+

17

57

81-100

05

+

18

100%

101-120

0.1

+

17

2076

T Pag 1

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Date

Invented by

Dat

Deena Polay

$$2 \mid 16 \mid 95$$

Recorded by

Appl. No. 09/558,421

Book No.

2-3-95 121

age No.	at my point added to reaction	Time point	* put 3rd H ₂ O/4
1. experiments			
J. Soler of 1-20-95	Run#		mix 1st 1X
2x R26 0.1% TN (Turner/NP40)	1-3	2 (0.05%)	= 48ul mix
0.2% BT Brj	4-6	Turner 20/NP40	20+2 X Enrg
0.2% TX Triton	7-9		gives 1/2 = 50
0.01% TN	10-12		
0.02% BT	13-15		
0.02% TX	16-18		
1.0% TN	19-21	(.04%)	
2.0 BT Brj	22-24		
2.0 TX	25-27		
No detergent	28-30		
(1.1X)	31-33	3.64	
(5X) → dilute 1/2.5 = .04%	34-36	2	
2x R26 0.1% + Enrg	37-39	1	
2x TFI 0.1%	40-42		
2x Vent buffer	43-45		
5ul			
dil = 0.04% /ul	46-50	2	

Reaction on 11
 this page 2-3-95
 152 3-9-95
 167 4-4-95
 36, 10 5-26-95
 52, 10 5-27

10' 74°C, N₂ / 10' 74°C
 spot 40 ul on 6 FC
 solution used 10' 74°C buffer 0/9-20-94 (P55)
 new stocks

Test Run mix	Mix P.W
3 EKBT 15% /ul 1-31-95	
no dil	2 48ul
1/125	2
EP9407	2
1/125	2

made new mix with stock shown in
 red on P120 and repeated experiment
 on 2-3-95 - results on next page (P122)

incorporation!

added 5ul of #12 (5X) into 12ul Tag dil buffer P55, 7

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Sarah A. Bolamp

Date

2/10/95

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Date

 2-1-95
 2-3-95

To Page No.

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122

Project No. _____

Book No. Avp

TITLE unit/pt

Relative
to Tray

Tray = 5

u/l x N

From Pr

1E	1	8410.00	}	8819	.037		.03	
	2	9136.00						
	3	8912.00						
	4	7465.00	}	7952	.033		.03	
2E	5	8664.00						
	6	7728.00						
	7	7737.00	}	7580	.032		.03	
3E	8	7235.00						
	9	7769.00						
	10	7579.00	}	6778	.029	✓	.02	
4E	11	(3001)00						
	12	6178.00						
	13	7484.00	}	7812	.033		.03	
5E	14	7833.00						
	15	8119.00						
	16	6228.00	}	6566	.027	✓	.02	
6E	17	6715.00						
	18	6755.00						
	19	8215.00	}	7824	.033		.03	
7E	20	8743.00						
	21	6514.00						
	22	7996.00	}	8413	.035		.03	
8E	23	8661.00						
	24	8581.00						
	25	7644.00	}	7533	.031		.03	
9E	26	6981.00						
	27	7976.00						
	28	4900.00	}	4989	.021	} no detector looks low can try + detector in unit assay	.02	
10E	29	4647.00						
	30	5419.00						
	31	7509.00	}	7702	.032		.03	
11E	32	6923.00						
	33	8674.00						
	34	8196.00	}	8075	.034		.03	
12E	35	7970.00						
	36	8060.00						
	37	8015.00	}	7442	.031		.03	
13E	38	7358.00						
	39	6954.00						
	40	8055.00	}	8479	.035		.035	
14E	41	8359.00						
	42	9023.00						
	43	7844.00	}	7611	.032		.03	
15E	44	7351.00						
	45	7638.00						
	46	9312.00	}	9580	(0.04)		.04	
	47	9496.00						
	48	9290.00						
avg	49	9726.00	}				by definition	
25	50	10073.00						
lat	51	58661.00						
	52	60427.00						
		2nd	ave = 59544 ⇒ 1,488,600 cpm/50μRxn					
		2nd						

ave = 57544 \Rightarrow 1,478,600 cpm/50 λ Rxn
37.2 cpm/pmol

To Page No

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Deanna Polanco

Date
2/16/95

Invented by

Recorded by

Date

2-3-95

Project No. _____

Book No.

TITLE

32P Z3mer dephatation reaction complete

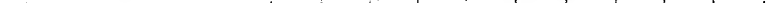
From Page No. Index # 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22

5x wings
P. 125

#	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	1	10		
4ml																					✓	✓	4	4

$\psi(0, t) = \frac{\text{primes}}{\text{primes}/1}$
 $34P23 \text{ nur } 0.26 \frac{\text{primes}}{\text{primes}} \times 0.6 \rightarrow \checkmark$

(Mund as p. 75)

Vent pol 2nd  2 2

0.51 / ml
diluted in vent storage
and dilution buffer
from NEB

50% glycerol		2.8	✓	✓	2.8
H ₂ O	13.4	10.6	✓	✓	4 1.2

$\nu_f = 20$ 70°C $20'$ $*340 \text{ } \lambda_{\text{comping}} \rightarrow 10 \text{ K} \lambda$

add 10 μ l cycle seq stop

(13 Rxns) cocktail preheat tubes to 70°C

(A) 7.81 (E) 7.81 then add Vent for 30 min 12 min

174.2 137.8 20 μ l Rxn has 0.16 pm primer = 18 pm primer

≈ 182 $\overline{182}$ 0.0 Unit Var at 100,000 $\mu/\text{mg} \approx$
1-10 #11-20 MW $\approx 100,000$

and 23 which gets 2 μ l of $\Rightarrow \approx 0.1 \mu\text{mol sol} / \text{inst}$
 $= 10.002 \mu\text{mol sol total sol}$

Ventil buffer

0.002 pmol/mol

$$0.38 \text{ pmol arcs} / 1.002 \text{ pmol mol} = 192 \text{ arcs/mol}$$

(a 20% with a 25mg unsealed)

OH 7.5	1.0 Tris	0.1 μ l	.3	
7 pmol primer / 1) P75		0.6 μ l	1.8	2.4 μ l 2.37 pmol primer

in 13 ml pipette + standard, $0.2 \mu\text{g/l}$
 $= 0.084 \text{ pmol/l}$ 9.3 27.9 $\frac{2.05}{0.78} \text{ pmol circles}$

19 ml 30 50°C, 5' cool down

To Pag	No
--------	----

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Dennis C. Polk

Date 2-17

Inv nted by 

Dat
2-10-95

age N .

1/5 Vent dil buffer

1/5 Vent dil buffer

Vent dil buffer

dilute 1/5 with 10mm

CF

50 mM Tris HEPH 7.4

Tris HEPH 7.5

20 mM

1 mM DTT

0.2 mM

0.1% NP40, Tween 20 each

0.02

50% glycerol

10%

100 mM KCl

10 mM

run 16% PAGE plus new reactions on 2-13-95

16% PAGE see P 144, 1

24 25 26 27 28 29 30 31 32 33

Vent buffer

2 2 2 2 2 2 2 2 2 2

#1 P125

(Vent buffer)

heavy PPO-83

20 mM

x #10 P125

(Heavy mix)

run (P126)

0.6

0.077 pmol circles / μ l

0.01 μ l

0.1 μ l

glycerol

15.4 15.4 13.4 13.4 8.6 8.6 13.4 13.4 11 4.2

2 2 2 2 2 2 2 2 2 2

2.8 2.8

15.4 15.4 13.4 13.4 8.6 8.6 13.4 13.4 11 4.2

2 2 2 2 2 2 2 2 2 2

2.8 2.8

15.4 15.4 13.4 13.4 8.6 8.6 13.4 13.4 11 4.2

2 2 2 2 2 2 2 2 2 2

2.8 2.8

15.4 15.4 13.4 13.4 8.6 8.6 13.4 13.4 11 4.2

2 2 2 2 2 2 2 2 2 2

2.8 2.8

15.4 15.4 13.4 13.4 8.6 8.6 13.4 13.4 11 4.2

2 2 2 2 2 2 2 2 2 2

2.8 2.8

15.4 15.4 13.4 13.4 8.6 8.6 13.4 13.4 11 4.2

2 2 2 2 2 2 2 2 2 2

24 25 26 27 28 29 30 31 32 33

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24 25 26 27 28 29 30 31 32 33

2 2 2 2 2 2 2 2 2 2

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2 2 2 2 2 2 2 2 2 2

Read & Understood by me,

serena Polak

Date

2/16/95

Invented by

Recorded by

Date

2-10-95

To Page No.

went to 50 with constant get 2000-2200 \checkmark 8.7 cm/hr

get ~ 2.4 cm/hr or need 3 hr

40% Acrylamide 200g
0.8% Bis 4g
H₂O

70°C, 12'

7% PAGE

start 1700 V at 1:45 pm
set 2 P Watts, 15 mAmp

run 1-10

11-20 pri

21, 22

24-32 pri

10 empty

big plate

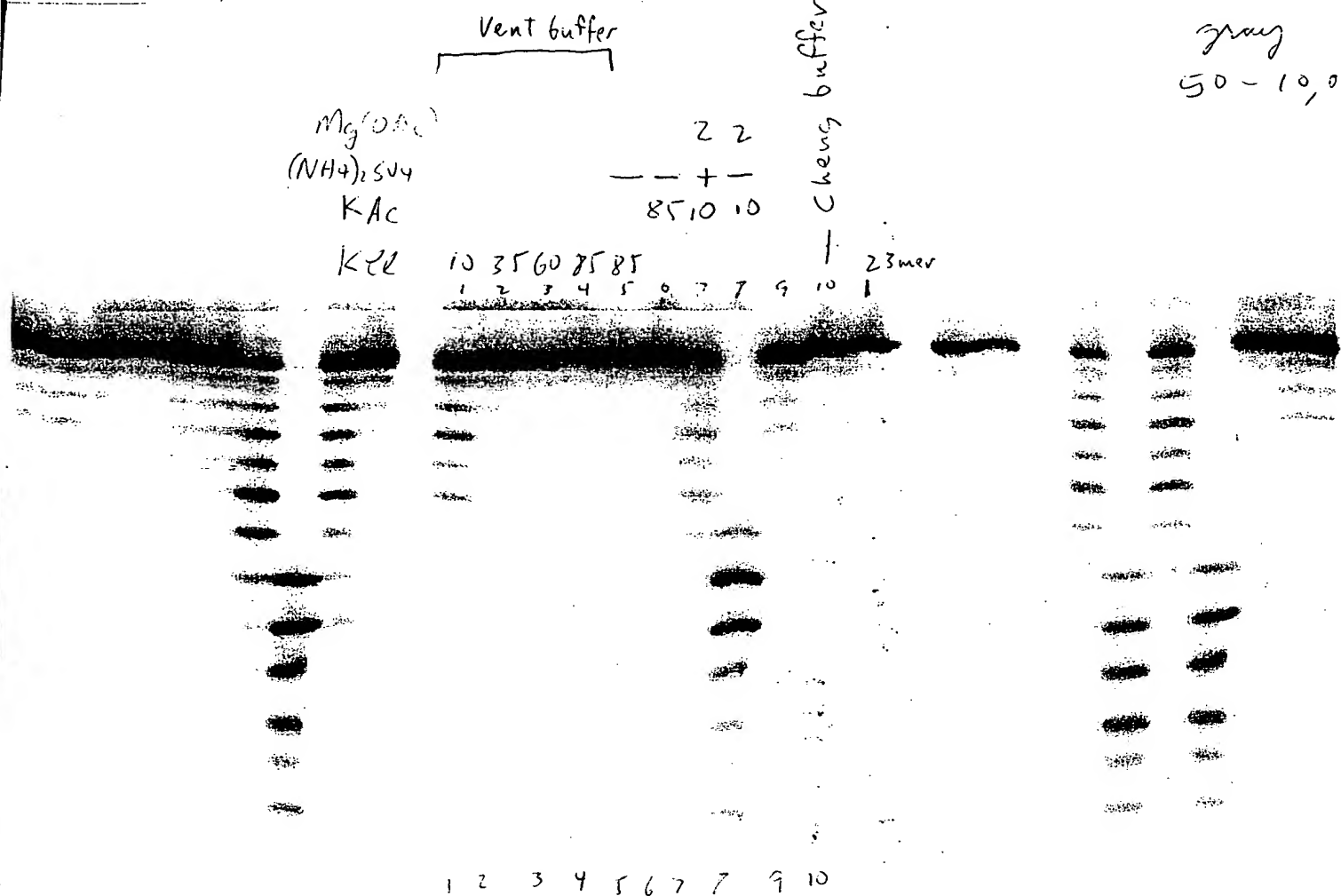
Project No. _____

Book No. _____

TITLE _____

128

From Page No. _____



Results:

- #1, 10 KCl + MgSO₄ is same as KOAc, MgOAc - get degradation if K ≤ 1
- 1-5 increasing ionic strength eliminates degradation. #5 also 85 mM KAc same as KCl 85 mM
- 8 leave out (NH₄)₂SO₄ get best result degradation of all (don't have (-)(NH₄)₂SO₄ for 10 mM KCl and MgSO₄ only 85 mM this result also consistent with ionic strength effect
- 9 substitute Tricine for Tris in Vent buffer has no effect
- 10 complete Cheng buffer - no degradation can be fully explained as due to 85 mM KAc - see # 4, 5 - 85 mM KCl or KAc & degradation in Vent buffer

Witnessed & Understood by m ,

Dat

Invent d by

Dat

T Pag

[Signature]

2/16/95

R c rd d by

2-13-95

- 02/14/95 - 06:45 pm

1.00x Counts

49.97



10000.00

D

Vent buffer
 NEB RL 5x G-TM RL
 .02 .2 .02 .2 .02 .2 .02 .2 .2 .2
 Vent (NEB) 23-mp19
 Cheng G-TM 23-mp19
 23 mer
 :Units
 Vent

23 mer is strongly
 protected when annealed
 to m13 ssDNA

To Page No. _____

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130

Tne vs Tag Project No. _____
 Book No. _____

TITLE

effect of KCl on pol on M13
 and primer degradation

From Page No. _____

(1) (2) (3) (4) (5) (6)

50 75 100 150 75 100

10 X Tag PCR buffer	8ul	✓	✓			
KCl 0.5M	4 8	✓	✓			
32P 23. m p19 (0.064 pmol 23. m p19)	8ul	✓	✓			(0.064 pmol)
10 mM 4dNTPs	1.6ul	✓	✓			200
MgCl2 50 mM	2.4ul	✓	✓			1.5
Tag 0.4 u/l	2 2 2					0.8
Tne 0.8 u/l	2 2 2					1.6
H2O	58 54 50 58 54 50	✓	✓			0.2 u

preheat tubes to 70°C, start with 2 ul pol VP = 80 ul

remove 10 ul at 1, 2, 5, 10 min to 5 ul cycle seq stop

* rTag EKBT1 1-31-95 5 u/l } both diluted in Tag dil buffer
 Tne 5 u/l A. Goldstein

32P 23 mer same as P.75 (0.267 pmol 23 mer / l)

32P 23. m p19

32P 23. m p19 0.267 pmol 23 mer / l	15.8 ul	14.2 pmol 23 mer tr
M13 m p19 0.2 ug / l (0.084 pmol circle / l)	50 ul	(4.2 pmol circle
1 mTns 7.5	0.6 6.6 ul	0.064 pmol 23.1
		use 1 ul / 10 ul re u

To Page 1

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Deena A. Goldstein

2/16/95

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2-15-95

Primer degradation (see PFO)

Project No. _____

Book No. _____

131

25-27 28-30 31-33 34-36 37-39 40

Tag N	(7)	(8)	(9)	(10)	(11)	(12)	
vent	8 th	8 th	8 th	8 th	8 th	8 th	✓ Does DMSO have any
Tag PCR buffer			8 th	8 th	8 th		✓ 2% DMSO
23 mer	1.91					1.6	✓ (0.64 pmol 23 mer / 10 ³) (= 6.4 nM primer)
1/2 50 mM	—	—	2.4				✓ (note Vent buffer has 2 mM MgSO ₄)
5 mM KCl				4	8		
0.8 M NaCl	2						
66	64.4	69					✓
		63.7	59.7	55.7	66.5		

heat to 70°C, remove 10 µl at 2, 5, 15

15 min only, total 40

pol / circles

0.1 unit Tag = 0.005 pmol (per 10 µl Rxn)

0.064 pmol 23 mer / 10³ (= 0.464 nM total / 10 µl)

4 pmol circle
5 pmol pol

0.012 3 ends / pol molecules

Expected units

0.1 u Tag gives 1 nM at / 30'

have 0.464 nM at 10³ reaction volume

need ~14 min to replicate all DNA at least 1 unit / 30' (but not sure M13 gives same units)
1 min would be ≤ 500 at extension at unit value rates

compared to PCR

Tag / time

- This would be 0.5 units / 50 µl PCR
- 6.4 nM primer (20 10³ times than 10 nM primer)

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Seena a Polamp

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Date

2-15-95

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132

From Page No. _____

100 bp ladder cut 10070-015

10 μ l H₂O (vortex)

1 μ l 10 mCi/ml ³²P dCTP

15' 37°C \rightarrow 10 \times 0.2 m EDTA

get total $> 10^7$ cpm

load 0.2 μ l

$\frac{(20 \times \text{total})}{(20 \times \text{volume})}$

10^7 cpm \rightarrow 5000 cpm/ μ l

after 10 μ l EDTA

put

20 μ l

10 μ l

30 μ l

(Rxn + EDTA)

cycles seq stop

$\geq 300,000$ cpm/ μ l

10,000 cpm/ μ l

30

load 1 μ l

T Pag N

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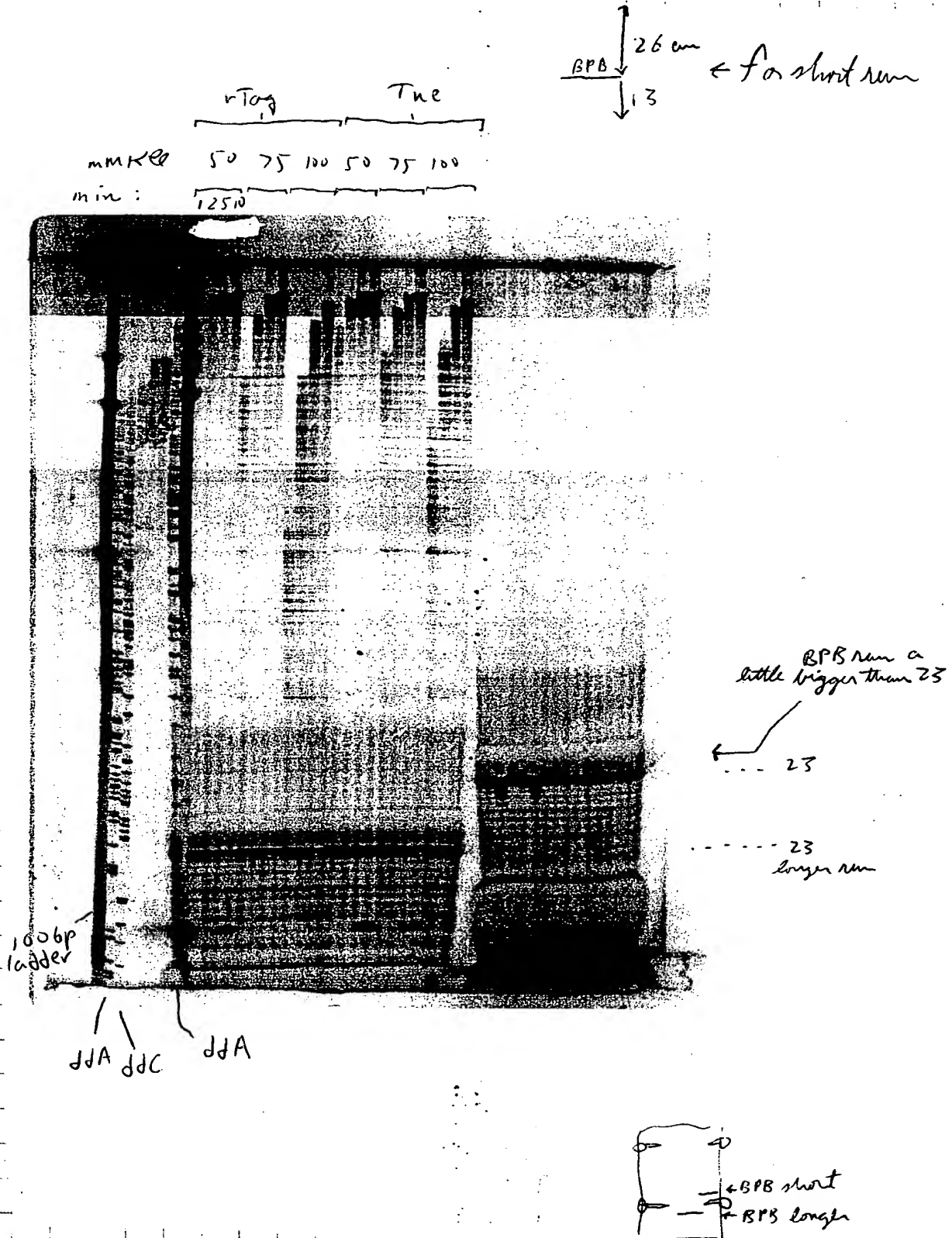
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 Book No. _____ TITLE _____

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Book No. _____ TITLE _____

From Page No. _____

primer 560826 (23mer with terminal A instead of G called "AC")

74.6 nmol total
746 \times H₂O

CP = 100 pmol primer / μ l (= 100 μ M primer)

Kinase

23mer "AC"	(100 μ M primer)	2 μ l	✓	✓	200 μ l
5x Kinase buffer	100 pmol 23mer / 1	8	✓	✓	23mer
32P γ ATP 10mCi / μ l (3.3 μ M ATP)	PNK 1 μ l / \times	20		✓	66 μ l
H ₂ O		2			
		40 μ l	✓	✓	

30', 37°C \Rightarrow 60°C, 5'

CP = 5 μ

use 2 μ l / 50 μ l PCR
for 200 nM primer

note 1 unit T4 Kinase converts 1 nmol ATP / 30' at 37

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[Signature]

2-2095

R c rd d by

B k No.

137

19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
x Tox PCR buff										5.1										→										✓																																																			
"AC" 1 p136 (15 μM)										2.1										→										✓										Cf = 0.2 μM 230																																									
20 0.5 M										- 1 2 3 4 5 - 1 2 3 4 5										✓																																																													
The 5 u/l →										2 μl →																				(4 units total)																																																			
dilute to 2 u/l																																																																																	
7, to 20 u/l																																																																																	
while to 0.2 u/l →										2 μl →																				(0.4 units)																																																			
Ag 2 50 mM										10.5 μl										2 μl →										✓										Cf = 1.5 mM																																									
H2O										59.5 53.5 51.5 50.5 49.5 48.5 47.5 46.5 45.5 44.5 43.5 42.5 41.5 40.5 39.5 38.5 37.5 36.5 35.5 34.5 33.5 32.5 31.5 30.5 29.5 28.5 27.5 26.5 25.5 24.5 23.5 22.5 21.5 20.5 19.5 18.5 17.5 16.5 15.5 14.5 13.5 12.5 11.5 10.5 9.5 8.5 7.5 6.5 5.5 4.5 3.5 2.5 1.5 0.5										✓																																																													

$m, M, K, C_A = 50, 60, 70, 80, 90, 100, 50, 60, 70, 80, 90, 100$

70°C, remove 10 μ l to 5 μ l stop at 20, 60, 120 min

Results on P13T

To Page No._____

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3/16/95

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2-21-85

TITLE 33 news matched and mismatched

GAATC

20 μ M primer

20 μm primer

(5.3 $\mu\text{mol total}$)

✓

(6.68 μmol)

✓

7

30' \rightarrow 5'

1	Amul
---	------

+

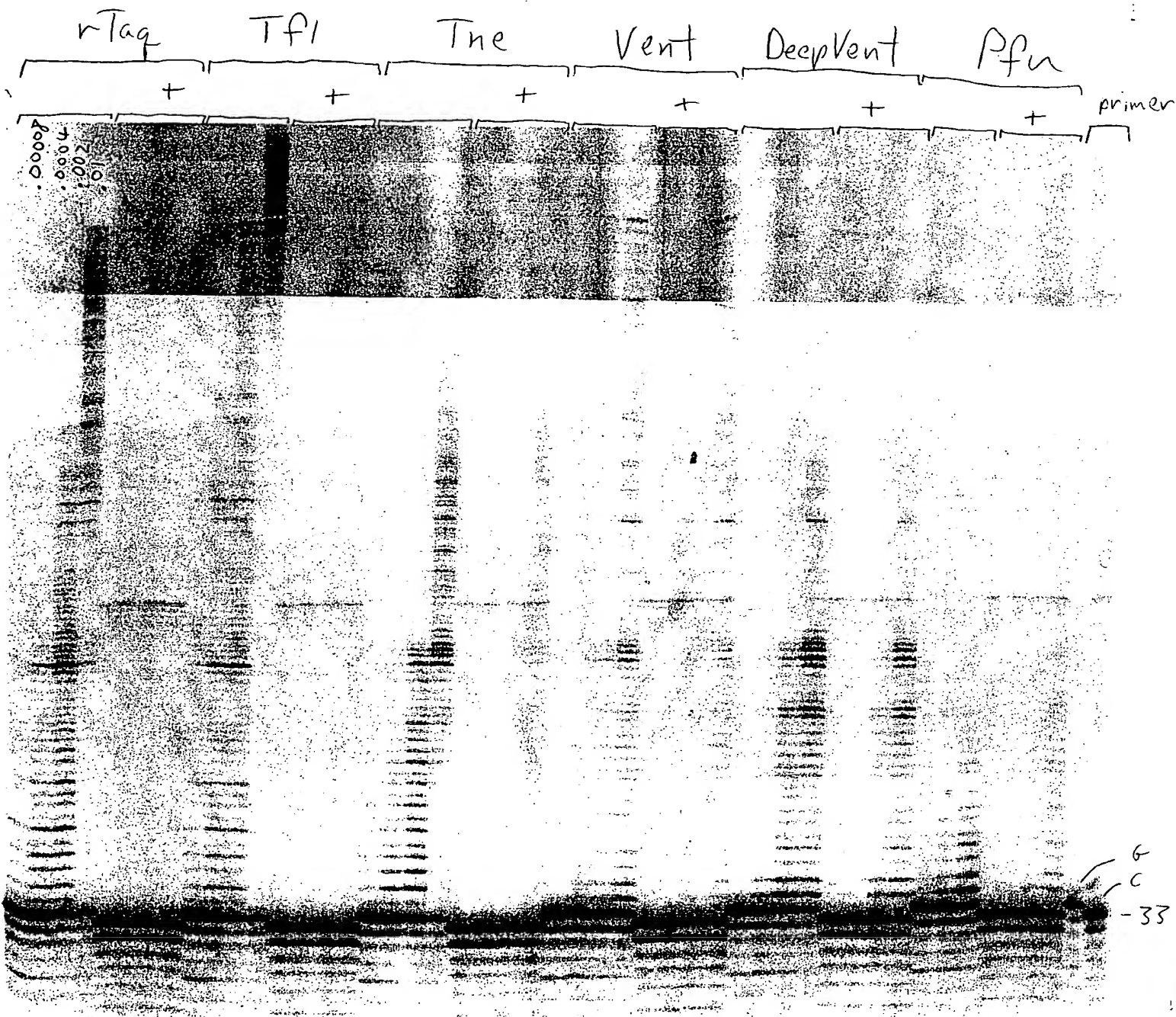
5. 6

7

5', 90°C cool slowly

$$= .06 \text{ pmol present in } 20 \mu\text{L}$$

Spec



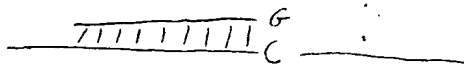
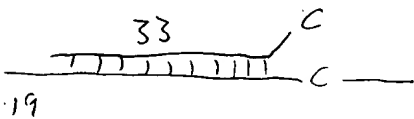
circles/mol molec

56

282

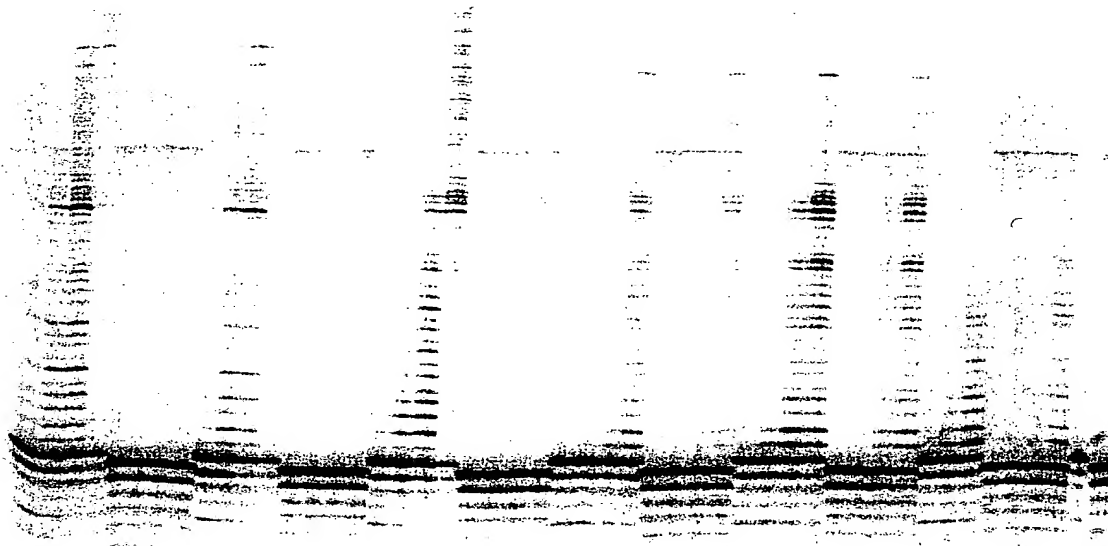
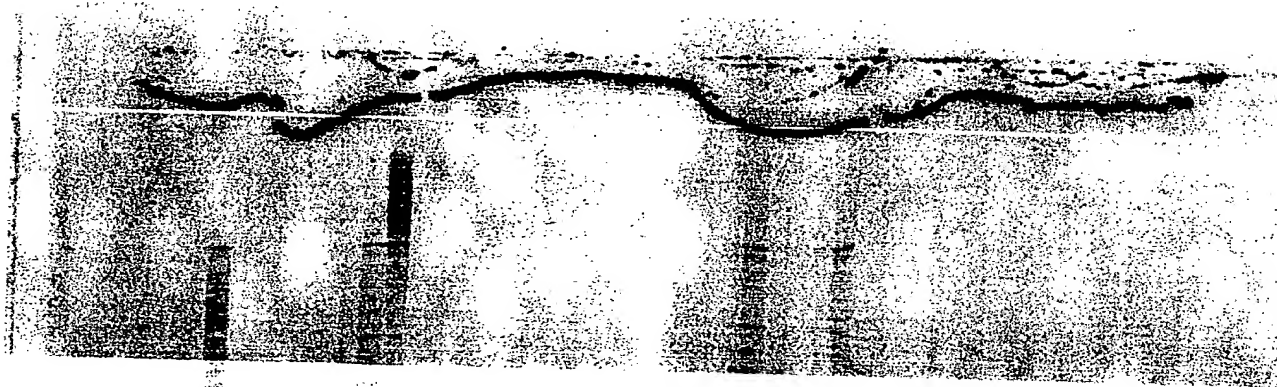
1410

7050





16% PAGE 33 watts, 4 1/2 hr
 XC went 21.7 cm
 33 mer migrated 25 cm
 XC runs as a 40 mer



was
in as 39 cm gel)

XC →

33 →

XC →

33 mer →

6 hr

25

14

could run XC to ~ 30 cm

le. 33 mer went
 25 cm of 39 cm
 gel long the

Tag No._____

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Date _____

Leandra Polanco

$$3 \overline{) 1695}$$

R c r d d by

2-7447

From Page No. _____

³²P correct. mp19 (P.138)³²P mix. - mp19. (P.138)

10mM dNTPs each

50mM MgCl₂

10x PCR buffer

10x Vent buffer

10x Pfu buffer

H₂OrTag .00008 μ l

-31-93 .0004

.002

.01

Tfi .00008

-orienter .0004

-15000A .002

.01

The .00008

.0004

.002

.01

Vent .00008

.0004

.002

.01

DeepVent .00008

.0004

.002

.01

Pfu .00008

.0004

.002

.01

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20-24-55

[illegible]

2 min 70°C
killed with 60 µl agarose stop

note: 0.03 pmol 3' ends / Rxn
for 0.00008 units (lowest level):

$$\frac{(10 \times 10^{-9} \text{ nmol/min}) (1.00008 \text{ u})}{30 \text{ min}} = 0.33 \text{ pmol}$$

∴ expect only ≈ 1 nt addition for each primer in 1 run (bases are even distributed and process of 1).

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General Polanco

$$3 \mid 16 \mid 95$$

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12-24 sj

Project No. _____

Book No. _____

TITLE

Repair of 3' mismatch
for TFI \pm Vent and rTag \pm DV, Pfu Tr

142

From Page No. _____

*

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22

32 P33 mis-mplg
(P138 encl 0.01 pm primary/1)

2

10 mM JNTPs

5x Chug complete (4/14/95) $\sqrt{4}$

0.4

5x chugach

H₂O

$\sqrt{13}$ 12 \rightarrow 13 12 \rightarrow 13 \rightarrow 13.4 12.4 \rightarrow 13.4 12.4 \rightarrow 12.6 \rightarrow

TFI 0.1 μ /1

$\sqrt{1}$ 1 \rightarrow

1 μ /1

$\sqrt{1}$ 1 \rightarrow

Vent .002 μ /1

$\sqrt{1}$ 1 \rightarrow

.01 μ /1

$\sqrt{1}$ 1 \rightarrow

.05 μ /1

$\sqrt{1}$ 1 \rightarrow

rTag 0.5 μ /1
5 μ /1

0.2 \rightarrow

0.2 \rightarrow

Deep Vent .002 μ /1

.01 .005

.05

1

1

1

1

1

1

1

1

1

Pfu .002 .005
.01 .005
.05

Tne .022 .01
.01 .05
.05 .01

(F=20)

preheat all reactions to 70°C, start by addition of
3' P33 mis-mplg, add 10 μ l cyd seq stop at 2 minutes

rTag, Tne TFI use Tag dil buffer
Pfu, Vent, Deep Vent use NEB Vent dil buffer

T Pag N

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Rid

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2-27-95

15 Rxw

[illegible]

To Page No.

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Date

Inv nt d by

Date _____

Recorded by

2-5-51

146

Project No. _____

Book No. _____

TITLE Δ KAc. Effect on pol and lxs, Tne v

From Page No. _____

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

5x Cheng, (no KAc
no DMSO
no Glycerol)
(at 5x = 100 mM Tricine pH 8.7,
5 mM Mg(OAc)₂)

✓ 4

KOAc 0.2 M

✓

0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

33 correct. mpA (same as
p13P. 0.06 pmol acid/λ)

✓ 2

32P 33 correct 5 μM primer
(as was done for "Ac" on 13.6)
10 mM 4 dNTPs
H₂O

✓ 0.4

✓ 11.6 10.6 9.6 8.6 7.6 6.6 5.6 4.6 3.6 2.6 1.6 11.6 10.6 9.6 8.6 7.6 6.6 5.6 4.6 3.6

Tag 0.001 μ/λ

2

Tne 0.004 μ/λ

2

Tne 2.0 μ/λ

V_f = 20λ

70°C, 5'

* 33 correct has
same 5' end as 23mer sequence primer

To Page N

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Deborah W. Zhang

3/16/95

R cord d by

[Signature]

3-1-95

Page No. 27 28 29 30 31 32 33

→ ✓

2 3 4 5 6 7 8 9 10 ✓

ul → ✓

12	11	10	9	8	7	6	5	4
2	2	2	2	2	2	2	2	2

JX Chung on P 79:

20 mM Tris HCl pH 7.7

1.2 mM MgOAc

80% glycerol

20% DMSO

plus KOAc which is varied

 $C_f \approx 200 \text{ mM}$ from 0-100 mM in this experiment.

→

→

70°C, 60'

start 11.8

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148

Project No. _____

Book No. _____

TITLE 10x PCR same as P140
is Tne inhibitory at T units ?

From Page No. _____

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
10x Taq PCR buffer	✓	2																			
³² P 3300000 mp19 (P146) (see P138)		2																			
50 mM MgCl ₂	✓	0.6																			
10 mM DTPA	✓	0.4																			
H ₂ O	✓	14											→ 13					→ 14			
rTaq																					
0.25 u/l	✓	1																			
0.5 u/l	✓	1																			
5 2	✓			1																	✓
5 3	✓			4																	
5 4	✓			.6																	
5 5	✓			.8																	
Tne																					
0.25 u/l										- 1							1				
0.5										1							1				
5 2											1							1			
5 3												4							4		
5 4												.6							.6		
5 5												.8									
Taq storage buffer	✓			.6	.4	.2					✓	.6	.4	.2			1			✓	.6 .4

preheat to 70°C, add 2 ³²P 3300000 mp19 for 30 sec
kill with 10 μ l cyclo sig stop solution
with 10 mM extra EDTA → 50 CF = 20 mM EDTA in stop

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3-3-95

Pr j ct No. _____

B k N . _____

149

ag N . _____

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Project No. _____
Book No. _____

TITLE AatII #1

³²P Kinase

150

From Page No. _____

see p 136 for ↑ [primer]

10 μ M AatII #1
5x Kinase
³²P ATP 10 mM
PNK 1 μ /l

5 ✓
4 ✓
10 ✓
1

(33 pmol ATP)

at IX
5 mM MgCl₂ 55 mM Tris
50 mM KCl

2 μ l

(\Rightarrow now its 2.5 μ M primer)

37°C, 30' \Rightarrow 80°C, 5'

↓

mix back into cold primer
at 5 cold to 1 hot primer

³²P AatII #1 2.5 μ M

13.3 20

(2.5 μ M)

cold AatII #1

16.6 25

(10 μ M)

30 45 μ l

(6.67 μ M)

(MgCl₂ = 2.2)

use 1.5 μ l / 50 μ l PCR for 200 nM
(adds 0.067 mM MgCl₂ to PCR (f))

Ayoob R. used in 14 PCR's

remove 10 μ l from each PCR to 5 μ l stop (ops)
and store at -20°C over weekend.

Result: Ayoob R.
did PCR's with The

note smear (see EtBr stain
(P151 photo) is not hot:
• primer ("AatII #1") is not
needed for smear

To Page

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Date

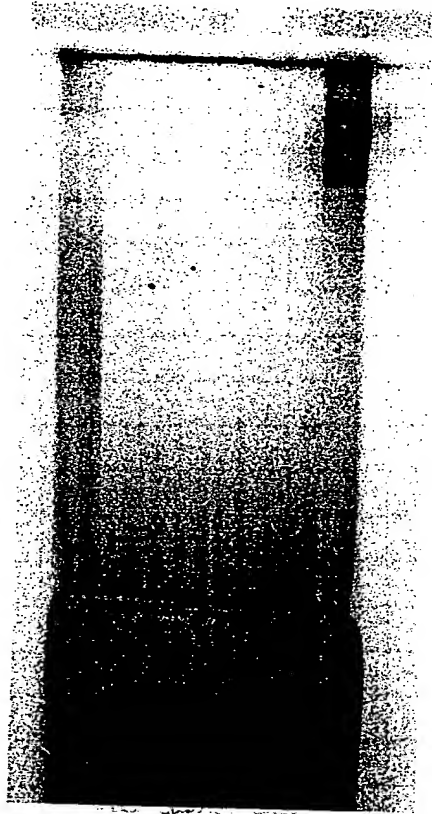
3-3-95

ag N _____

10% *agave* 180 V
1 2 3 4 5 6 7 8 9 10 11 12
1 1 2 3 3 4 4 5 6 6 7 7

0.5 x TBE

(lost 2nd, 5th)



To Page No. _____

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3-7-95

152

Project No. _____

Book No. _____

TITLE

Unit assay for stability of vTag in
PCR mix. Repeat of assay on p 121

From Page No. _____

This assay is 33 days after the first assay of 2-3-95.

carry out all assays with exact same procedure
of p 120 - 122, same Mag PL, TAPS, Kcl mix of p 121same stock of 'activated DNA', same 5' u/pl vTag stock
on p 121 → (of 1-31-95)
(3' p d TP is a new stock of 10 mc/ml on 3-10-95)

T Pag N

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3/16/95

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Date

3-9-95
3-8-95

relatd to Tag \Rightarrow u
and $\frac{1}{1}$

Pr j ct No. _____

B k No. _____

153

Exhibits

Activity					activity
1	23165.00	} 24896	.031	.037	84
2	26508.00				
3	25014.00				
4	24738.00	} 24616	.031	.033	94
5	23608.00				
6	25502.00				
7	23947.00	} 23577	.030	.032	94
8	24449.00				
9	22336.00				
10	19450.00	} 19801	.025	.029	86
11	20001.00				
12	19953.00				
13	21103.00	} 22158	.028	.033	85
14	20211.00				
15	25159.00				
16	19309.00	} 18853	.024	.027	89
17	18318.00				
18	18933.00				
19	22404.00	} 23332	.029	.033	88
20	25483.00				
21	22108.00				
22	20542.00	} 23507	.029	.035	85
23	27602.00				
24	21776.00				
25	22624.00	} 22051	.028	.031	90
26	23813.00				
27	20017.00				
28	10829.00	} 11703	.015	.021	(70)
29	12483.00				
30	11798.00				
31	23967.00	} 24527	.031	.032	97
32	25056.00				
33	24557.00				
34	26587.00	} 25000	.032	.034	93
35	23432.00				
36	24980.00				
37	25401.00	} 24694	.031	.031	100
38	24104.00				
39	24576.00				
40	25123.00	} 25962	.033	.035	94
41	25545.00				
42	27217.00				
43	24143.00	} 23703	.030	.032	93
44	23491.00				
45	23474.00				
46	30440.00	} 31731	(-.04)		
47	31721.00				
48	30572.00				
49	32938.00	} 17377	.022		
50	32985.00				
51	17357.00				
52	17994.00	} 144943			
53	16781.00				
54	144943.00				
55	145358.00				

note #10 is not authentic. so collect det be
at .01 to 20/MP4 each in Reaction mix

To Pag No. _____

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Date

3-10-85
3/16/85

Invented by

R. Polay

Date

3-9-85

Recorded by

Test of rule to use 1/600 Toz sol
between 20-40 min after mixing

ag N - Standard Toz units assay as per 120-120

10 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

be # 1,23 4 5 6 7 8 9
tube # 1,23 4 5 6

21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40

10 11 12 13
7 8 9 10 11 12 13

41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 70 2hr 3hr

14 15 16 17, 18
14 15 16 17, 18

make a standard 1/600 dil °

1797 µl Toz dil buffer
3 µl 5 u/l Toz
Vortex 5"

use immediately in triplicate for reactions 1, 2, 3 at
0, 20 sec and 60 sec
also # 1797 20 sit on ice + 2 3 hr before 10.1
EDTA (is not used at 74°C to see if any activity at 0°C)

To Page No. _____

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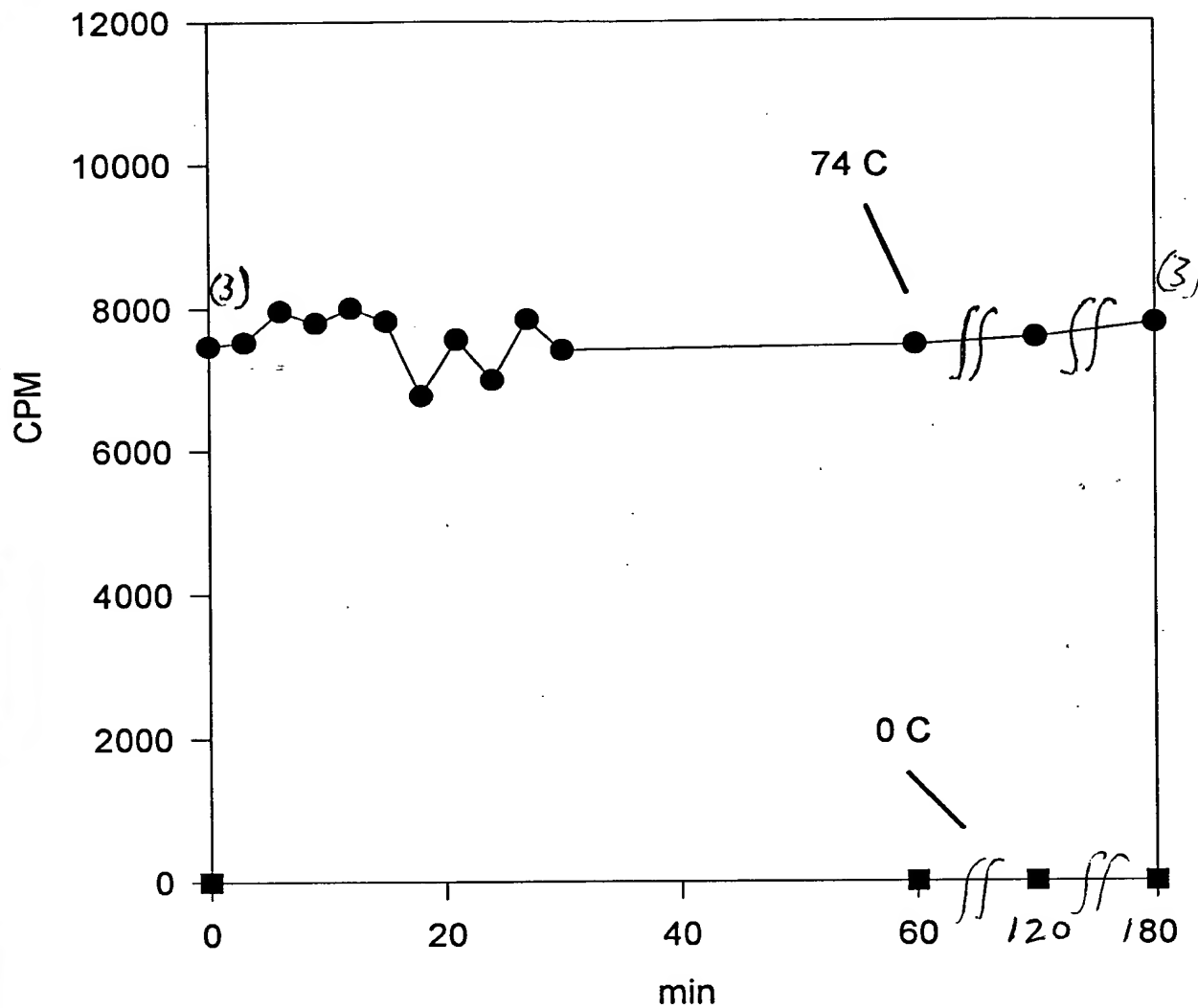
3-15-95

Project No. _____

Book No. _____

TITLE _____

Time allowed before assay of Taq dilution



SAM	CPM
1	738
2	845
3	705
4	770
5	809
6	802
7	820
8	796
9	692
10	764
11	733
12	801
13	760
14	970
15	765
16	784
17	750
18	828
19	827
20	27
21	66
22	40
23	59

Witnessed & Understood by me,

Deena Polansky

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4/4/95

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3-15-95

Tet stock / streak T+1 clones

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Block No. _____

Exhibit L-54

Appl. No. 09/558,421

157

Page No. _____

0.4g Tetrazoline Sigma crystalline (not salt)
40 ml ETH

Amp/Tet plates

have BBL Amp plates (100 µg/ml) Vol. ~ 15 ml agar
to make 50 µg/ml Tet spread

15 µl 10 mg/ml Tet on each - let sink in ≥ 30 min
25 50 µg/ml Tet in 35 ml agar plates

streak out cell (glycerol) stocks of AR

sup 94-95 for stock names

grow at 30°C O/N

start 2 ml O/N from single colonies 3-21-95
of each in will grow, 100 µg/ml Amp, 50 µg/ml Tet

3-22-95

inoculate 0.4 ml of each O/N into 35 ml will grow
+ 100 µg/ml Amp, 50 µg/ml Tet

shake at 30°C starting at P. 3

To Page No. _____

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Carolina Polansky

Date

4/4/95

Invented by

Recorded by

Date

3-20-95
3-21-95

Tet stock / streak T+1 clones

Project N

Block N

Exhibit L-55

Appl. No. 09/558,421

157

Page N

0.4g

Tetracycline

Sigma crystalline

(not a salt)

40 ml

ETOH

Amp/Tet plates

have BBL Amp plates (100 µg/ml) Vol ~ 15 ml agar

To make 50 µg/ml Tet spread

75 µl 10 mg/ml Tet on surface - let sink in ≥ 30 min

25 µl 50 µg/ml Tetr in 5 ml agar on plate

streak out cell (glycerol) stocks of AR

SUP 94-95 for stock names

grow at 30°C O/N

start 2 ml O/N from single colonies of each in wide grow, 100 µg/ml Amp, 50 µg/ml Tet

3-22-95

inoculate 0.4 ml of each O/N into 35 ml wide grow + 100 µg/ml Amp, 50 µg/ml Tet

shake at 30°C starting at 8:30

To Page No.

Read & Understood by me,

Deborah Polansky

Date

4/4/95

Invent d by

Recorded by

Date

3-20-95
3-21-95

Tet stock / streak T+1 clones

Project No. _____

Exhibit L-56

Appl. No. 09/558,421

Block No. _____

157

Page No. _____

0.4g

Tetracycline

Sigma crystalline

(not a salt)

40 ml

ETOH

Amp/Tet plates

have BBL Amp plates (100 µg/ml) Vol ~ 15 ml agar

To make 50 µg/ml Tet spread

Typical 10 mg/ml Tet on water - let sink in ≥ 30 min

25 µg/ml Tet in 50 ml agar on plates

streak out cell (glycerol) stocks of A.R.

SUP 94-95 for stock names

grow at 30°C O/N

start 2 ml O/N from single colonies 3-21-95
of each in which grow, 100 µg/ml Amp, 50 µg/ml Tet

3-22-95

inoculate 0.4 ml of each O/N into 3.5 ml which grow
+ 100 µg/ml Amp, 50 µg/ml Tet

shake at 30°C starting at 8:30

To Page No. _____

Used & Und restod by m ,

Date

Invented by

Date

Deanna Polansky

4/4/95

Record d by

3-20-95
3-21-95

From Page No. _____

3-22

30°C
 Start 8:30
 12:30 .274
 2:00 .770

ASSD

↓ 42°C, 15 min

↓ 1 hr 37°C

3-23

extract and 55°C heat for FrI is
 same as p 95 and p 115, 6

pol assay is same as PRT except add just
 2 µl FrI' / 97 µl Rxn cocktail
 and remove three points

array 1, 2, 5 µl of FrI' in 50 µl
 Top unit array (using TFI buffer system)
 for 5 min at 74°C

3-23-95

12-15-94

106	(100)	64
107 H	87	92
108 H	86	(100)
152	83	59
151	56	95
202	20	26
109	2	11

To Page No. _____

Witnessed & Understood by me,

Deanna Polak

Dat

4/4/95

Invnted by

Recorded by

Dat

3-23-95

Proj ct No. _____

Book No. _____ TITLE _____

158

From Page No. _____

3-22

30°C
Start 8:30
12:30 .274
2:00 .770

↓ 42°C, 15 min

↓ 1 hr 37°C

3-23

extract and 55°C heat for FrI is
same as p 95 and p 115, 6

pol array is same as P95 except add just
2 µl FrI' / 97 µl Rxn cocktail
and remove three points
array 1 2 5 µl of FrI' in 50 µl
Top unit array (using TFI buffer system)
for 5 min at 74°C

	<u>3-23-95</u> %	<u>12-15-94</u>
106	(100)	64
107 H	87	92
108 H	86	(100)
152	83	59
151	56	95
202	20	26
109	2	11

To Page No

Witnessed & Understood by me,

Deanna Polak

Date

4/4/95

Inventor by

Recorded by

Date

3-23-95

3-23-90

Project No. _____

Book No. _____

TITLE

5% PEI stock

160

From Page No. _____

Same as P155, 6 except ~~use~~ instead
of using complete Tag ext buffer (P167, 3)
just use 50 mM Tris HCl pH 7.5, 1 mM EDTA

A = 50 mM Tris pH 7.5 275 ml
1 mM EDTA

(5526 UABR) PEI 50% 50 ml

ster $\geq 30'$

adjust pH to 7.4 with HCl
add A to $V_f = 500$ ml

T Page 1

Witnessed & Understood by me,

Date

Initiated by

Date

Diana Bolamp

4/4/95

Received by

3-24-95

grow 2L TFI-106

Proj ct No. _____

Exhibit L-59

Appl. No. 09/558,421

B ok N . _____

161

ag N . _____

make 2x LB (in 40 g / L of LB broth base
eg as per P 119, b for D. + o k

make 20 ml O/N of TFI-106
in LB + 100 µg/ml Amp, 30 µg/ml Tet
(Mony L uses 15-20 µg/ml Tet)

10 mg/ml

Ampicillin (Sigma A-9518)

2 g

H₂O

200 ml

filter sterilize

inoculate 10 ml O/N / 1L LB

start shaking at 30°C at P: 20 AM
start 8:20 AM ASD
12:30 PM 0.567

induce each at 42°C, 15' - by rapidly
bringing up to 42°C in hot tap water bath
and then 42°C in water shaker 15'

↓
37°C 1 hr in air shaker
cool in ice water bath

end 1 hr 37°C at 2:05 and 2:35 respectively

OD₅₅₀ final = 0.812 ⇒ approx 5.1E 653 45 min
recovered 5.64g cells

To Page No. _____

Used & Understood by me,

Date

Inventor by

Date

Deena Polanco

4/4/95

Recorded by

3-26-95

3-27-95

Project No. _____

Book No. _____ TITLE _____

162

is buffers for 50g TFI prep

From Page No. _____

follow v Tag PRP 91342. PRP * except for w
2m KCl in
buffer
see.

2L
buffer B

1L
buffer C

1M K phos monobasic ✓ x 34.2

17.1 ml

1M K phos dibasic ✓ x 15.7

7.9 ml

glycerol ✓ x 160

80

KCl ✓ x 7.46g
(50mm) d

149.12
(2m)

EDTA 0.5M x x 0.4ml

0.2 ml

BME 14.5M ✓ x 700 μ l

350 μ l

H₂O 2L

1L

buffer C is 2ml
here in order to de
elution point - m
in Tag PRP C.
700 mm KCl

Witnessed & Underst od by m ,

Deanna Bolano

Date

4/4/95

Inv nt d by

R c rd d by

Date

3-27-95

T Page N

AmSO₄ optimization for TFI
(can see p 22, 7 for Tag)

Project No. _____
B k No. _____

Exhibit L-61
Appl. No. 09/558,421

163

ag N

3.64 g TFI cells (P161)

18 ml Tag ext buffer (P167,3)

sonicate

heat treat 75°C, 30 min

PEI

adjust to 200 mM NaCl
Vol = 20 ml so add 1.33 ml NaCl 3M

add 5% PEI (P160) to C_p = 0.4%
stir 15 min (1.7 ml 5% PEI)

Centrifuge SS 34 15' 15 K

recover 17 ml supe = Fr I' / PEI

start 11:30 AM
stir AmSO₄ in 15', spin
SS 34 15 K, 15 min

	2 AmSO ₄	at salt
1 Fr I' / PEI	2.45 g	25
2	.433 g	30
3	.51	35
4	.51	40
5	.527	45
6	.527	50
7	.544	55
8	.561	60
9	.561	65
10	.578	70

To Page No. _____

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Date

Inv nted by

Dat

Deena Polanco

4/4/95

Record d by

3-29-95

164

Project No. _____

Book No. _____

TITLE

Pol assay of AmSO₄ supes

From Page No. _____

*array 2 ml of 1/100 dil of each supes in 48
ml R x n mix (P-120) for 15 min at 74°
kill with 10% EDTA
spot 40λ*

Bradford

*I / PEI
AmSO₄ 25%*

*ml
20*

ASIS

mg/ml

*To Protein
remaining*

*.412
.450
.430
.449
.425
.405
.370
.347
.340
.278
.242*

*(0.39)
0.40
.38
.40
.38
.36
.33
.31
.30
.25
.21*

*100
102
97
102
97
92
85
79
77
64
54*

I / PEI / 70%

20/20

BSA 1 mg/ml

*1
2
4
6
8
10*

*.604
.105
.176
.263
.382
.474
.546*

*AmSO₄
760*

CPM1

u/ml = 0.64

*0 1 (11483.00)
25 2 10706.00
30 3 11635.00
35 4 10329.00
40 5 7609.00
45 6 803.00
50 7 465.00
55 8 514.00
60 9 258.00
65 10 313.00
70 11 230.00
Blank 12 126.00
2λ 13 106668.00*

*(100)%
93
100
90
66
7
4
4
2
3
2
—*

⇒ 1,0980 u/17ml FRI

*3017 g cells
(3.64g cells used)*

*conclude 45% AmSO₄
bring down > 90% min*

⇒ 66.7 cpm

BSA

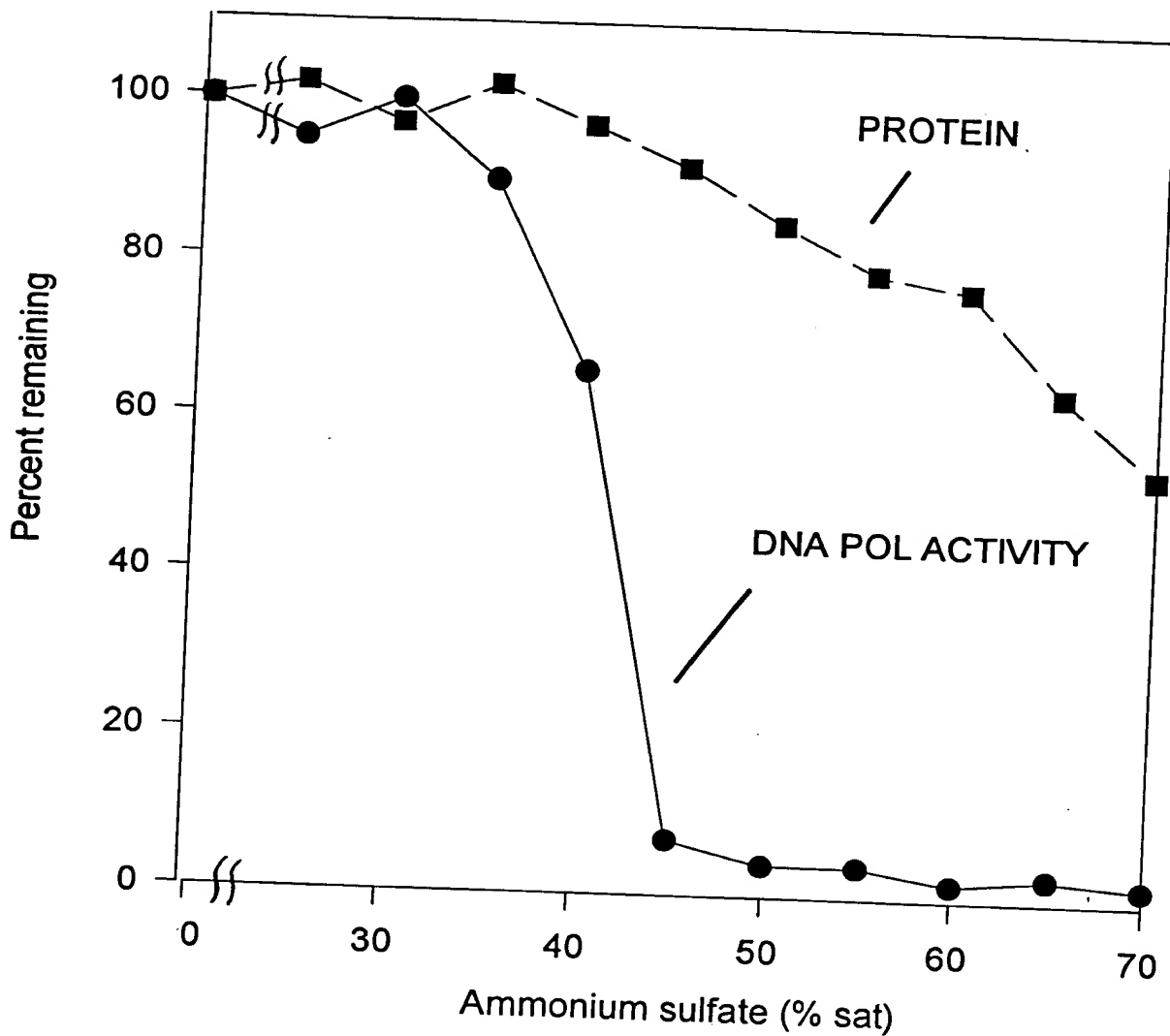
Project No. _____

Block No. _____

165

ag No. _____

Precipitation of Tfi DNA polymerase



To Page N _____

Issued & Understood by m ,

Date

Investigated by

Date

Recorded by

166

Project No. _____

Book No. _____

TITLE from a 180 ml sepharose 200

From Page No. _____

in a Mannin 2.6 X K

1. make slurry of cold sepharose 200 - want a
1.5 X vol of paste vol

so 1.5 X 200 ml slurry = 300 ml

2. add 100 ml col buffer (buffer B p 162)
so vol now = 2 X paste vol

and reservoir and gravity flow (got 50 ml/min with effluent tube 2
below bottom of column and 100.
in reservoir) - $\sim 1/4$ vol vol/hr

bed volume ended up ~ 185 ml (2.6 cm X 35 cm)

5 well 25 ml (bed vol) of Blue sepharose (Ph
EL 65, in buffer B (P 162))

since dry swells 4 X 6.25 g

T Page N. _____

Witnessed & Understood by m ,

Deena Polap

Dat

4/4/95

Invent d by

Record d by

Date

4-31-95

Stability unit assay for Targ
 same as p121 and 122

Page N. _____

note stability study tube # 10 (unit assay # 27-30)
 get 0.010% Taren 20/10P40 each added to reaction
 by adding 0.5 ml of 10% stock

tube 51-56 =

Dextran	1.25 mg/ml	1 ml	Cf
	2.5	1	0.025
	5	1	0.05
	10	1	0.1
	10	2	0.2 mg/ml
	10	3	0.4
			0.8

51 19252.00
 52 18303.00
 53 18777.00
 54 18582.00
 55 17015.00
 56 17487.00
 57 267.00
 58 104554.00

→ 65.3 cpm/pmol

To Page No. _____

Issued & Understood by m ,

Erica Polak

Date

4/13/95

Invented by

Recorded by

Date

4-85

From Page No. _____

SAM

CPM1

u/ml assuming
 rTag in .04u/ml in 1/125861 ↓
 .032 .037 8604

1	13329.00		
2	14243.00		
3	14542.00		
4	14132.00		
5	13839.00	.032	.033 97
6	13367.00		
7	14361.00		
8	14576.00	.033	.032 97
9	14684.00		
10	11765.00		
11	12054.00	.027	.029 93
12	11446.00		
13	13666.00		
14	13091.00	.030	.033 91
15	12913.00		
16	10381.00		
17	10049.00	.024	.027 89
18	10787.00		
19	16428.00		
20	14956.00	.034	.033 103
21	15556.00		
22	15357.00		
23	14468.00	.033	.035 94
24	13489.00		
25	14348.00		
26	12027.00	.030	.031 97
27	13354.00		
* 28	9416.00		
29	8913.00	.021	.022 100
30	9177.00		
31	13920.00	.032	.032 100
32	13672.00		
33	13373.00		
34	14628.00		
35	13728.00	.033	.034 97
36	15178.00		
37	14616.00		
38	14209.00	.034	.031 109
39	15366.00		
40	14402.00		
41	14584.00	.034	.035 97
42	15003.00		
43	12819.00		
44	13391.00	.030	.032 94
45	13180.00		
46	16169.00		
rTag 47	18733.00	17463 are (.04u/ml) by definition	
48	18552.00		
49	16396.00		
50	12907.00		

*fn #10, we .022 u/ml on P153 then
 dat added as 0.000 point

To Page No.

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Date

Inv nt d by

Dat

D. C. R. Polans

4/13/95

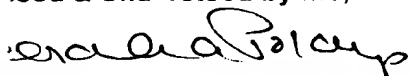
R c rd d by

4-4-95

ag N	P12 in other point	(P112)		
		1 month	2 months	4 months
1	.1% TN	84	86%	98
2	.2% BT	94	97	98
3	.2% TX	94	97	106
4	.01% TN	86	93	93
5	.02% BT	85	91	105
6	.02% TX	89	89	98
7	1% TN	88	103	104
8	2% BT	83	94	91
9	2% TX	90	97	99
10	No detergent	95	95	91 -
11	1.1X	97	100	94
12	5X	93	97	100
13	2x R2GE 0.1%	100	109	(35)
14	2x Tff 1.01%	94	97	89
15	2x Vent	93	94	97

To Page No. _____

Used & Understood by me,



Date

4/13/95

Invented by

Recorded by

Date

4-4-95

Project No. _____

Book No. _____

TITLE _____

Tf1 growth of 4-4-95

170

From Page No. _____

got ~ 0.6-0.8 g cells from 50ml samples taken at 0 1 2 3 4 hr post induction for 10L per of minimal media (001R) and the same for buffered rich (002R) plus 50ml at end (~4h post induction) for (002R) plus 114g bulk
chipped off 0.55g of bulk for 4hr 002R sample

Resuspended cells in Taz ext buffer (P167, 3)
add 25 ml ext buffer \Rightarrow 0.2 g cells/ml
sonicate 3 x 10 sec max setting microtip
microport 15 min, supe = Fr I

90°C 5 min
microport 15 min = supe = Fr I'

62.2 $\frac{\text{CPM}}{\text{pmol}}$
see P167

pol assay is 2 μL of 1/100 and 1/500 dil of Fr I'

hr	cpm	pmol	μL	Graded Assay μL	mg/ml	μg
0	874.00	-21	0.31	0.548	1.03	300
1	440.00					
2	6172.00		2.6	.677	1.28	2029
3	1538.00		1.8	.648	1.23	1467
4	5174.00		2.6	.670	1.27	2050
5	1058.00		2.1	.667	1.25	1675
6	6330.00		0.58	.639	1.21	314
7	1537.00					
8	5734.00		2.1	.672	1.29	1626
9	1206.00		1.7	.700	1.33	1282
10	1058.00		1.8	.641	1.21	1485
11	324.00		1.3	.734	1.27	1018
12	3961.00					
13	1227.00					
14	4250.00					
15	1009.00					
16	4730.00					
17	1046.00					
18	3435.00					
19	763.00					
20						

T Pag No.

Witnessed & Understood by me,

Deerana Polap

Date

4/13/95

Invented by

Record d by

Dat

4-5-95

114 g Thomas flc ~~cells~~
mini g granlin

Proj ct No. _____ Exhibit L-66
B k N . _____ Appl. No. 09/558,421

171

ig No. _____

cells 9504-02-767-03-002R
(4 hr after induction)

Follow rTag PRP Document # 91342. PRP

114 gram cells

450 ml Tag extract buffer (buffer A)
with fresh ^{5mm} BME + 50 µg/ml PMSE

~ 564 ml (30 ~ 0.2 g cells/ml)

one pass thru minigranlin 10,000 PSI

heat to 75°C ~~30~~ (~15')
in 40°C water bath

15 min more at 75°C → cool in ice slush

Adjust NaCl to 200 mM

have 550 ml Fr I' (ie after heating)

add 6.43 g NaCl

PEI adjusted to 0.4% by adding

47.8 ml 5% PEI pH 7.4 slowly, then
ster 15 min more

To Page No. _____

s d & Understood by me,

Ernest A. Polansky

Date

4/13/95

Inv nted by

Recorded by

Date

4-7-95

Spin 30 min in GSA 13,000 RPM

J Ammonium sulfate

Recovered 506.6 ml of Fr I' / PEI
want 47.5% $\text{Am}(\text{SO}_4)_3$ saturation

$$= 295.5 \text{ g / 1L}$$

so add 149.7 g to 506.6 ml Fr I' / P

add slowly, stir 30 min more

centrifuge GS-3, 2500 rpm, 60 min

— $\text{Am}(\text{SO}_4)_3$ pellet was coming off side of bottle
after 60 min spin
looks like pellet & solution

will try 2 hr at 15000 RPM in GSA

27000 g compared to ~12000 for GS-3

and smaller bottles (~150 ml / bottle in 4 bottles)

— result: pellets still floating

— collected ppts in filter and mixed into
32 ml of clear filtrate

— spin 30 min in SS-34 18K

and spin 1 ml of 32 ml total in microfuge for
init assay.

ag N

Try diluting 1:1 the suspended AmSO_4 ppt
+ Try ext buffer lacking glycerol (ie 50 mM
Tris HCl pH 7.5, 10 mM KCl) plus 47.5%
saturated AmSO_4 . ie, the only effect is
to reduce % of glycerol from 8% to 4%
to see if ppt will pellet better

Result: ppt floats in 4% and also no glycerol!
it does ~~not~~ sink in H₂O

Result:
see P 176 - cells induced only 1 hr don't
have problem of AmSO_4 pellets not sinking
must be too many lipids in cells used here
from 4 hr fermentation time point!

To Page No. _____

Read & Understood by m ,

Carla Delamp

Date

4/13/95

Invented by

Recorded by

Date

7-2-95

Project No. _____

Stability of Tay at room Temp

174

Book No. _____ TITLE _____

From Page No. — see P154, 3-13-91. Samples have been at room Temp 21
 assay same as P121, 152.

0.5 M

Taps 200 ml pH 9.3 (at room Temp)

(243.3 mW)

(Sigma T^{cat#} - 5130)24.33g + ~140 ml H₂O

2M KOH to pH 9.3

H₂O to 200 ml

trial # 1 - 30 is stability study 1E-15E in duplicate
 note tubes 19, 20 (no detergent) gets 0.5ul of 1% Tween 20/R
 24, 24 in the reactions.
 (ie sample is stability study)

To Page No.

Witnessed & Understood by m ,

Dana A. Polap

Dat

4/13/95

Invented by

R c rded by

Dat

4-11-95

Results of #114
 Crack TFI borne as P

Project N _____
 B ok N _____

g N _____

	SAM	CPM1	ave	$\frac{a}{x}$	from TIME	of P121 values
	1	26896.00	26508	.04%	1.00	
	2	27150.00			1.00	
	3	26135.00			1.00	
	4	25462.00			1.00	
	5	26896.00			1.00	
1	6	22094.00	22048	.033	.037.00	89
	7	22002.00	22955	.035	.033.00	106
2	8	22874.00			1.00	
	9	23036.00	22335	.034	.032.00	106
3	10	21345.00			1.00	
	11	23325.00	17637	.027	.029.00	93
4	12	17420.00			1.00	
	13	17853.00	19840	.030	.031.00	91
5	14	19189.00			1.00	
	15	20491.00	14229	.021	.027.00	78
6	16	14064.00			1.00	
	17	14394.00	20655	.031	.033.00	94
7	18	19638.00			1.00	
	19	21673.00	20245	.031	.031.00	89
8	20	22693.00			1.00	
	21	17798.00	18271	.028	.031.00	90
9	22	17031.00			1.00	
	23	19511.00	—	.022	.022.00	0
98 100 N	24	804.00			1.00	
	25	710.00	18729	.028	.032.00	88
11	26	17770.00			1.00	
	27	19687.00	18729	.028	.032.00	88
12	28	166725.00			1.00	
	29	170523.00	19521	.030	.034.00	97
13	30	19772.00			1.00	
	31	20070.00	19376	.029	.031.00	83
14	32	21891.00			1.00	
	33	16862.00	22789	.034	.032.00	106
15	34	24156.00			1.00	
	35	21422.00	1454.00		1.00	
BK00	36	1454.00			1.00	
21	37	134586.00			1.00	

To Pag No. _____

ed & Understood by m , <i>renera Poking</i>	Date <i>5/1/95</i>	Invented by <i>[Signature]</i>	Date <i>4.11.95</i>
		Recorded by	

176

Project No. _____

Book No. _____

TITLE

Crack TFI same as P 171

From Page No. _____

~~These cells only grown for post induction~~

cells are 9504-10-767-03-003R
grown 4-11-95

resuspended 110 g cells in 440 ml (at room
ext buffer (P167, 3) but no detergent
10,000 PSI on minigun, 1 pass
Bring to 75°C in 90°C water (~10 min)
75°C for 15 min more.
cool in ice slurry

Add NaCl to 200 mM Cf

Fr I vol = ~~510~~ 510 ml
add 5.96 g NaCl

add PEI (5% stock pH 7.4) to Cf = 0.

(used 0.4% last time (P 171) but want to get as
as much DNA as possible

add 50.4 ml 5% PEI to 510 ml Fr I + N.

⇒ Cf = 0.45% add PEI dropwise and
stir 15 min more

spin GSA 13,000 RPM 30'

recovered 49.5 ml sup (= Fr I' / 1

Witness d & Underst od by me,

Dat

Inv nt d by

Dat

T Pag N

Domena Polans

5/1/95

R c rd d by

4-13-95

P116 continued
Experiment done on P. 123

Pr j ct N .

Exhibit L-70

Bo k N .

Appl. No. 09/558,421

117

19 N .

Still Needed 3

cut with Ord I to see if full length Sac Z is present
(assuming either Af I II or Aa I II recognition region
had a point mutation generator). Therefore the "410" and "465" bp

miniprep # 54, 58, 64, 73, 87, 98, 103, 108, 113, 120

plus Aa I II, Af I II

cut with 55 I to see if R1 site in MCS was
a point mutation (or very small deletion
(see on P107 at bottom) resulting in the "90mers"

miniprep # 3, 29

Recut with 17 μ l miniprep and load 30 μ l?

^{25 μ l reaction}
to try to resolve the "No results"

miniprep # 20, 39, 71, 74, 75, 76

To Page No. _____

sed & Understood by me,

Date

2/16/95

Invented by

R c rded by

Date

1-31-95

see a Boland

From Page No. _____

resuspended entire AmS₂ pellet in buffer B (P.)
 added 3 ml to ~ 1 ml pellet.
 triturate

spin SS34, 13 K RPM, 5 min

add ~ 200 μ l buffer B to pellet
 respin \rightarrow 200 μ l buffer B more

need to microfuge 15 min to clarify

$V_f = 3.5$ ml (~~ca~~ 1.9% of 180 ml G100 col)

Load on 180 ml sephacryl 200

elute with $\frac{1}{2}$ col vol/hr buffer B (ie 1.5 ml/min)

note mol started coming off
 column ~ 98 ml

98 ml / 180 ml col vol \approx 54% col vol

Witnessed & Understood by me,

Deanna Polans

Date

5/1/95

Invented by

Record d by

Date

4-18-95

To Page N .

PAGE 179 OF NOTEBOOK WAS BLANK

Project No. _____

Book No. _____

TITLE

Standard
TfI unit assay

From Page No. _____

mix used by epicenter same as Tag unit assay (P120)
except only 160 μ g DNA/ml instead of 500 μ g
in Rxn

A
0.5 M Tris pH 9.3 5.00 μ l
1 M MgCl₂ 60 μ l
3 M KCl 5.00 μ l
V_T = 2,060 μ l

"TfI Rxn mix"

A 229 μ l ✓

10 mM dNTP 6.67 μ l ✓
3.7 mg/ml DNA 144.2 μ l ✓
10 mM γ -³²P dCTP 6 μ l ✓
H₂O 2754 μ l ✓
3.2 ml

use 48 μ l / 50 μ l reaction

T Page N

Witnessed & Understood by me,

Dat

5/1/95

Invented by

Dat

Record d by

Debra Polansky

4-1-95

	SAM	CPM1	pmol	u/ml	total units
PET	1	4110.00	163	2.45	1.21×10^6 units in 495 ml
spend	2	6087.00		562.9	1.28×10^6 in 3.5 ml Aves 04 resuspended
4	3	308.00			fraction
5	4	356.00			pool 7-12
6	5	678.00			= 18 ml total
7	6	3373.00			
8	7	8181.00			
9	8	11817.00			
10	9	9111.00			
11	10	8925.00			
12	11	5943.00			
13	12	2583.00			
14	13	1385.00			
15	14	773.00			
16	15	351.00			
17	16	299.00			
18	17	304.00			
19	18	245.00			
20	19	407.00			
21	20	2651.00	105	1.58	(expected only 1 u/ml in stock from epicenter)
22	21	358.00			
23	22	818.00			
24	23	60259.00			

37.7 cpm/pmol

for ~~Fr 5~~⁷⁻¹² = average of ~ 8000 cpm for 17 μ l
 $\Rightarrow 47.7$ u/ μ l \Rightarrow [859,000 total units / 17 μ l]
 or $\sim 72\%$ recovery from Fr I' / PEI

Project No. _____

Book No. _____

TITLE

Blue sepharose

182

From Page No. _____

load pooled sepharose 200 fractions #7-12 (18 ml V to
on 20 ml Blue at 0.35 ml/min (~1 col vol)
wash 5 col vol o/v at 0.16 ml/min buffer
gradient is 400 ml vol 50 mM - 1 M KCl
(use buffer B-C - p162)
at 3 col vol/min = 1 ml/min, 6 ml fraction

Buffer

im Tris pH 7.5
0.5 M EDTA
Glycerol
3 M
KCl

* D

200 ml ✓
1.6 ml ✓
640 ml ✓
2.8 ml ✓
29.7g ✓

PC

(50 mM KCl)

E

25 ml 12.5 ✓
0.2 ml 0.1 ✓
40 ml ✓
0.2 ml 0.175 ✓
14.9g 74.5g ✓

LK

500 ml

2 M KCl

(*note buffer D is
75 mM KCl in Tag Pop 71342)
but only 50 mM here)

enter "2" to set to bank 2
then 5

HOD 5 BANK 2

.00 CONC XB A 0
.00 CONC XB n n
.00 ML/MIN 1
.00 P1/P2/TET

400 —
400 —

still 1

a

To Page N

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Date

5/1/95

Invented by

Recorded by

Date

4-15-95

Fig N. — pool Blue fractions 24-32 based on UV profile

$V_f = 54$ ml

Dialyze against 5L buffer D (PIB2) O/N
recovered ~ 60 ml

Conductivity

10 μ l in 1 ml H_2O

buffer D
via vol effluent
to equilibration O/N

10.1 μ S = 10.1 mS
9.8 μ S = 9.8 mS

Dialyzate

9.9

9.9 mS

(can see P 41 where results are similar)
for TOG

To Page No. _____

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serena Polansky

Date

5/1/95

Invented by

[Signature]
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Date

4-20-95

PAGE 184 OF NOTEBOOK WAS BLANK

Heparin AF (20ml vol)

Proj ct No. _____

B ok No. _____

185

g N. — Equilibrate O/N with buffer D, P182 (50 mM KCl) → see P183 for conductivity of col effluent
 Load ~ 60 ml dialysate (P183) at 0.67 ml/min
 = 2 vol vol/hr (as done on P11 for rTag)
 (1 min/min)

wash ~ 1 vol vol 0.67 ml/min

for gradient want to make it fairly flat for first try
 of TFI on Heparin. ϕ

Gradient:

50-700 mM KCl (= 0-35% pump B since
 E is 2 mM KCl)

20 vol vol = 400 ml, 4 ml/hr (so 100 fractions total)
 run at 2 vol vol/hr
 ϕ need 10 hours for whole gradient

rTag comes off Heparin ~ 400 mM KCl (see P 46)
 ϕ might see TFI ~ 6 hr from start ~ late afternoon
 if TFI same as Tag

(loading done ~ 10:25 AM, wash 30 min (= 1 vol vol)
 gradient start ~ 11 AM

10D 5 BANK :

.00 CONC XB
 .00 CONC XB
 .00 ML/MIN
 .00 PORT.SET
 .00 PORT.SET
 .00 VALUE.POS
 .00 VALUE.POS
 .00 CONC XB
 .00 ML/MIN

To Pag No. _____

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erica Polay

Date

5/1/95

Inv nt d by

Rec rded by

Date

4-20-95

[illegible]

To Page No._____

s d & Understood by me,

Date

Invented by

Date _____

Здравствуй

5/1/95

Recorded by

4.21-5J

From Page No. _____

			<u>p.mol</u>	<u>w/pl</u>	<u>average w/pl</u>	<u>fraction</u>	<u>total units</u>
38	1	321.00	12		1.9		
39	2	526.00	20	3.0	3.0		
40	3	1566.00	60	9.03	9.9 ave		
41	4	928.00		10.7		4ml	39600
42	5	513.00					
43	6	326.00					
44	7	3904.00		22.52	21.9 ave		
45	8	1849.00		21.3		4ml	87600
46	9	1346.00					
47	10	792.00					
48	11	5730.00		33	40.5	4ml	162000
49	12	3486.00		40			
50	13	1668.00		38			
51	14	1117.00		51			
52	15	5064.00		29			
53	16	3156.00		36	41.3	2.68ml	110684
54	17	1890.00		43			
55	18	1239.00		57			
56	19	6029.00		34			
57	20	3974.00		45.8	43.8	2.68	117384
58	21	2233.00		51			
59	22	969.00		44.7			
60	23	4489.00		25.5			
61	24	2775.00		22.5	35.6	2.68	95408
62	25	1960.00		45			
63	26	858.00		39			
64	27	2156.00		12.4			
65	28	1056.00		12.1	12.3	2.68	32964
66	29	843.00					
67	30	364.00					
68	31	847.00			4.9		
69	32	465.00			2.7		13000
70	33	4246.00					
71	34	2441.00	93.9	14.0		54ml	756000
72	35	3795.00				(mol fr 24-32)	
73	36	2266.00				60ml	
74	37	165.00		13.9		after dialysis	751000
30 100% recovery from dialysis							

32.5 w/pl/pmol

(occasionally w/pl thin
 Opt. is extracted
 pmol fraction)

Witnessed & Und rsted by m ,

Deena R. Sharp

Dat

5/1/95

Invent d by

R c rd d by

Dat

4-21-95

T Pag 1

ag N	units	% recovery
I'/PEI	1,210,000	100%
monium sulfate	1,280,000	100
phacryl 200	859,000	71
ue sepharose	756,000	62
alysis	751,000	62
arin AF	666,000	55

To Page No. _____

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Date

Recorded by

Sandra Polay

5/1/95

4-21-95

190

Project No. _____

Book No. _____

TITLE

grow λ pL sp6 plasmid
in host lacking TFI sp6 plas
but containing Tet resistance
plasmid with λ promoter and
sp6 gene

From Page No.

Tet stock

LB 50 ml
Tet 30 μ g/ml
add stab of cells \rightarrow 30°C shaking O/N
4-24
got no growth O/N ! \rightarrow as it just grew
slow - had cells 36 h

Repeat with M. luteus Tet and
at only 15 μ g/ml
after 36 h) grow O/N at 30°C
should dissolve "crystalline" TC in E
made fresh ~~TC~~ TC stock in H₂O first

13.9 mg Sigmastat 3258 Tetracycline
a little water
(it still doesn't go into solution)

100% ETOH (good stuff from Corning)
up to 27 ml
= 5 mg/ml store in foil, -20°C

inoculate 1 ml of ON #5 into 50 ml
can circle grow + ~~15 μ g/ml~~ 30 μ g/ml of fresh TC stock
shake at 30°C

start 8:30 stop 3 PM got 0.22 mg cells
to add 0.88 ml Taget buffer (P167, 3) (for 0.2 μ g/ml)

Witnessed & Understood by me,

Deena a Poling

Date

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Initiated by

Recorded by

Date

4-23-95

To Page N

Regeneration of columns

Proj ct No. _____

Exhibit L-76

Appl. No. 09/558,421

B ok No. _____

191

N. —

Blue sepharose

2 vol vol 6M Guanidini HCl
5 vol vol H₂O (immediately)
2 vol vol 20% EtOH for storage

Heparin Af

2 vol vol 4M urea
2 vol vol H₂O
2 vol vol 20% EtOH

(Co. 3 = 0.5M NaOH recommended)

sepharose S200

1/2 - 1 vol vol 0.4M NaOH

contact with vol = $\geq 1 \text{ hr} \leq 2 \text{ hr}$.

H₂O 2 vol vol

20% EtOH for storage

run 0.4M NaOH at 2 ml/min
for 45 min (= 1/2 vol vol)

(start 10:20 AM) H₂O for 3 hr at 2 ml/min
= 2 vol vol and NaOH only in
contact with column for
45 min x 90 min

20% EtOH 3 hr 0.2 ml/min O/N

To Page No. _____

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Date

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Date

sepharose S200

5/1/95

Recorded by

Y. 2855

192

Project No. _____

Book No. _____

TITLE _____

SDS gel for IfI prep

From Page No. _____

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

DHIOBRK2

Fr I P190

3λ

Fr I' (75°C, 30) P190

30λ

IfI Fr I 2.45 u/l (P190)

3λ

Fr I' 2.45 u/l (P190)

30λ

Am SO₄ resuspended

0.5

3.62 u/l

5200⁰⁰⁰ 50 u/l

3.5

Blue pool Fr 24-32

12.5

14 u/l (P190)

Hepain Fr #

39 3 u/l

5

40 9.9

5

41 21.9

5

42 40.5

5

43 41.3

5

44 43.7

5

45 35.6

5

46 12.3

5

47 4.9

5

IfI epimorph 1 u/l

30

cut TF31010A-502

2X sample buffer

30

H₂O

27-27-3027 17.525

load 15 ul MW standards

CTI cut 10064-012

run at ~29 mA

started 9:15 AM

T Pag N ..

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Deena Golap

Date

5/1/95

Invent d by

Record d by

Date

4-25-95

(180 ml)
47 cm Heparin AF column

Project No. _____ Exhibit L-78
Bo k N . _____ Appl. No. 09/558,421

1

ge No. _____

proved a 1.5 cm x 47 cm (180 ml) column
to try to separate the 2 peaks on P186-187, 9
flow rate is 0.204 ml/min by gravity.

gradient will be 50 mM - 400 mM
and 10 col vol = ~~1600 ml~~ 1600 ml
so gradient 1/2 as steep as P185, 9 : 20 ml col

pool fr 40-43 (14.7 ml total)
of Heparin (see P185-192, 9)

Dialyze ON against 1 L buffer D

(frms are ~ 300 mM KCl
so expect ~ 4.2 mM + 50 mM in buffer)
start gradient ~ 9:30 AM
gradient is

1600 ml (20 col vol)
50 mM - 400 mM KCl (was 50 mM - 700 mM on)

2 ml in 5 ml/min, so ^{13.3} 13.3 hr for gradient

4.5 ~~hr~~ min/frn = 9 ml/frn (20 frns total)

note 1.5 ml/min gave only 0.2 mPa (column
is definitely running with backpressure) but
2 ml/min still only ~ 0.2 mPa so will
use 2 ml/min \Rightarrow 1.5 col vol/hr
used 2 col vol/hr for 20 ml col P185, 9

To Page No. _____

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maera P ooy

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Date 4-26-95

4-27-95

From Page No. _____

expect protein to start coming off at $\sim 65\%$ of the
gradient. ie $\sim 1040 \text{ ml} = 8.7 \text{ hrs}$
or $\sim 6:30 \text{ pm}$

since pool started coming off at $13\% \text{ } \phi + 50 \text{ mm}$
 $= 410 \text{ mm}$

Comparison of 80 and 20 ml columns

col vol	20 ml	80 ml
col height	11 cm	47 cm
gradient vol	20 col vol	20 col vol
gradient stop	$\frac{35 \text{ mm KEE}}{\text{col vol}}$	$\frac{20 \text{ mm KEE}}{\text{col vol}}$
flow rate	2 col vol/hr	1.5 col vol/hr.

Therefore the new col is $4 \times$ longer, has $0.75 \times$
flatter gradient and is $0.75 \times$ slower flow rate
so hope to get better separation of 2 peaks see
on P 186-187, 9

THOD 5 BANK 2
 1.00 CONC XB C
 1.00 CONC XB C
 .00 ML/MIN 2.
 .00 PORT.SET 3
 .00 PORT.SET 6
 .00 VALUE.POS 1
 .00 VALUE.POS 2
 3.0 CONC XB 20
 3.0 ML/MIN 2.1

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Date

Deeven Polarp

5/1/95

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4-27-95

PAGE 3 OF NOTEBOOK WAS BLANK

Project No. _____

Book No. _____

TITLE

SOS gel on 80ml Heparin

From Page No. _____

I' DHIOBRK2
P190

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19

MW
150

I' TF1 P1P1

200

AmSO4 resw 362 μ /A

1.5

S200 50 μ /A

145

Blue

62

With Heparin frns

105

150

106

150

107

150

108

150

109

150

110

150

111

150

112

150

113

150

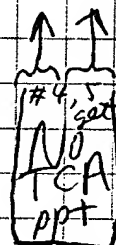
114

150

115

150

TF1 10+TF3 1010A-502



150

H₂O

100 100 - - 238 150

Kf = 300

15% TCA

300 - - 300

P 72, 9 and P 50, 7)

30' ice

1 x sample buffer

3550 ml - 400

31

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Deborah Polys

Date

5/1/95

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R c rded by

Dat

4-28-95

T Page N

PAGE 5 OF NOTEBOOK WAS BLANK

Storage buffer

Fr m Page No.

buffer F, G as per rtag 91342.PRP

make F, 4L first as follows.

make vol up to $\frac{3200}{2700}$ ml ($\therefore 80\%$ of V_f of

remove	160 ml	and add
	20 ml	Tween 20 (Pierce)
	20	NP40 (Pierce)
ml	<u>200</u>	

= buffer G

Take the remaining buffer up to V_f = 3200
for 1X buffer Fpool frn 105-114 of Heperan (P. 1-4)
= 90 ml vol (actually measured 87 ml)Dialyze against 2 buffer F, 5 hr
2 ml L O/N

Recovered 33 ml after Dialysis.

combine with 33 ml buffer G = V_f 66.

labeled:

TFI DNA pol in
storage buffer 4-30-95

store at -20

4.33 ml
sup B

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Deena R. R. R.

Date

5/1/95

Inv nted by

R c rd d by

Date

4-29-95
4-20-95

T Pag N

Storage buffer

From Page No. _____

buffer F, G as per rTag 91342. PRP

make F, 4L first as follows.

make vol up to $\frac{3200}{2700}$ ml (i.e. 80% of Vf of

remove	160 ml	and add
	20 ml	Tween 20 (Pierce)
	20	NP40 (Pierce)
ml	<u>200</u>	

= buffer G

Take the remaining buffer up to Vf = 3800
for 1X buffer Fpool frn 105-114 of Heperum (P. 1-4)
= 90 ml vol (actually measured 87 ml)Dialyze against 2 buffer F, 5 hr
2 ml C O/N

Recovered 33 ml after Dialysis -

combine with 33 ml buffer G = Vf 66.

labeled: TFI DNA pool in
storage buffer 4-30-95

stock at -20

4.33 ml
ACIPB

Witnessed & Understood by me,

Deena R. R. R.

Dat

5/1/95

Invent d by

Recorded by

Dat

4-29-95
4-30-95

To Pag N

Unit assays for H1

ag N	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
in storage buffer																											
4-30-95																											
sterile																											
250	2				2				2				2														
500		2				2				2				2													
1000			2				2				2				2												
2000				2				2				2				2											
lot 30809B																											
5 (see P12)																											
50																	2										
100																		1									
200																			2								
400																				2							
F310/10A-502																											
4-25-95 P12, 9																											
0.5 gel																											
50																					2						
100																						2					
200																							2				
400																								2			
4-25-95																											
0.5 gel																											
50																									2		
100																										2	
200																											2
400																											2
Rxn mix 48ul																											
20, 9)																											
unit assay																											
mix																											

74°C, 10', → 10μl 0.5M EDTA → spot 40μl on GFC

Tubes # 13-16 are same serial dilution as tubes # 1-4
made 3 independent serial dilutions in Tag dil buffer

To Page N _____

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Date

Invented by

Date

Researcher

5/1/95

Recorded by

5-2-95

From Page No. _____

	SAM	CPM1	$\mu\text{mol} - 200 \text{ BKG}$	$\mu/\mu\text{L}$		
dil 1	1	4167.00	102.8	3.85		
	2	2383.00		4.24	4.25 ave	
	3	1401.00		4.67		
	4	1060.00		(6.68)		
	5	4115.00		3.80		
dil 2	6	2316.00		4.1	4.38 ave	
	7	1394.00		4.6		
	8	846.00		5.0		
	9	4180.00		3.5		
	10	2416.00		4.3		
dil 3	11	1226.00		5.18	4.37 ave	
	12	888.00		5.5		
	13	4556.00		4.22		
Tag Rxn	14	2720.00		4.89	4.66 ave	
unt	15	1452.00		4.81		
(Jus) skd	16	1330.00		(8.28)		
	17	6287.00		1.17		
	18	4373.00		1.62		
	19	2428.00		1.73		
30709B	20	1302.00		1.87		
	21	13554.00		2.59		
	22	8692.00		3.3		
1010A	23	5151.00		3.8		
	24	3002.00		4.36		
	25	12756.00		2.44		
	26	8623.00		3.27		
1010A	27	5397.00		4.06		
	28	2964.00		4.30		
BKG-D	29	194.00				
= 200	30	96542.00				

5-2-ST label Hepair fm of 4-30-ST
at

4.33 units / λ

T Pag N

Witnessed & Understood by m ,

Deena Solari

Date

5/15/95

Inv nt d by

Rec rd d by

Date

5-2-ST

Endonuclease Qc

Project No. _____

Exhibit L-84

Book No. _____

Appl. No. 09/558,421

9

follow + Tag Qc: 10342. QCP

	22 Rxns	SS DNA Rxn mix	JS DNA Rxn mix	
PCR buffer		110 μ l	110 μ l	✓
M Tris HCl pH 8.4)				
20mM KCl				
2mM MgCl ₂		110	110	✓
174 (+) ssDNA 0.2 μ g/ μ l		110 μ l		✓
174 RF 0.33 μ g/ μ l			66.7	✓
all H ₂ O		666 990	703.3 990	✓

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
4.30.50	.48	.92	1.39	1.8	2.3												
3u/ μ l																	

spicula	.57	1.14	1.7	2.3	2.9
10A-502					
u/l (p8)					

crude xpt
I 4-13-95
il

1/10,000
1/1,000
1/100
1/10

no del
4.5 4 3.0 3.15 2.7 4.4 3.9 3.3 2.7 2.1 4 4 4 4 4 5 - ✓
5

7.0 il buffer
Vp=50 μ l 72°C, 3 hr (start 12:10 PM)

1-17 is 45 μ l SS DNA Rxn mix and #18-34 is JS DNA Rxn mix 45 μ l Rxn mix

To Page No. _____

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Date

5/15/95

Invented by

Recorded by

Dat

5-3-95

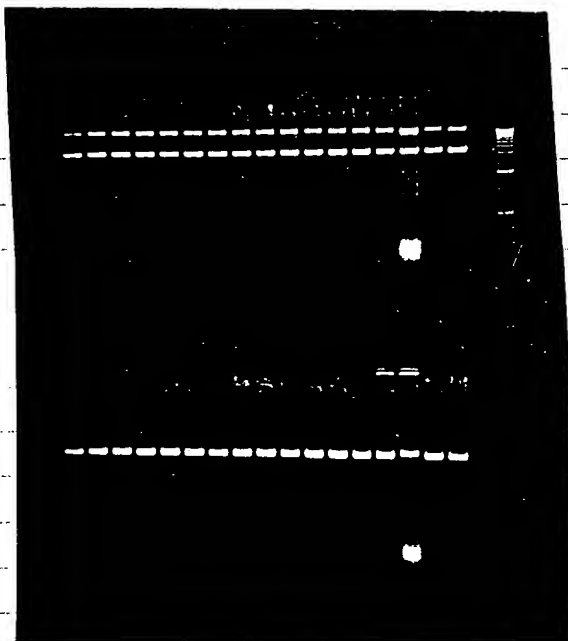
From Page No.____

units

CTI	TFI	Epicatech	crude ext
2	4	6	7
8	10	12	14

Blank / dil buffer blank

(relaxed) RFI \Rightarrow SC DNA \Rightarrow


$$\left\{ \begin{array}{l} ds \\ \text{endv} \end{array} \right.$$

} 551
end

note for RF \times substrate with positive control (F₁I)
there is a slight nick at in relaxed circles (ie nick
and immediate conversion to small fragments.
no nicking seen for ~~the~~ either ssDNA or dsDNA
substrates ~~by~~ using TFI or Epicenters.

To Page N

Witnessed & Understood by me,

Polarp

Dat

5 | 15 | 95

Invented by

Recorded by

Date _____

5-3-95

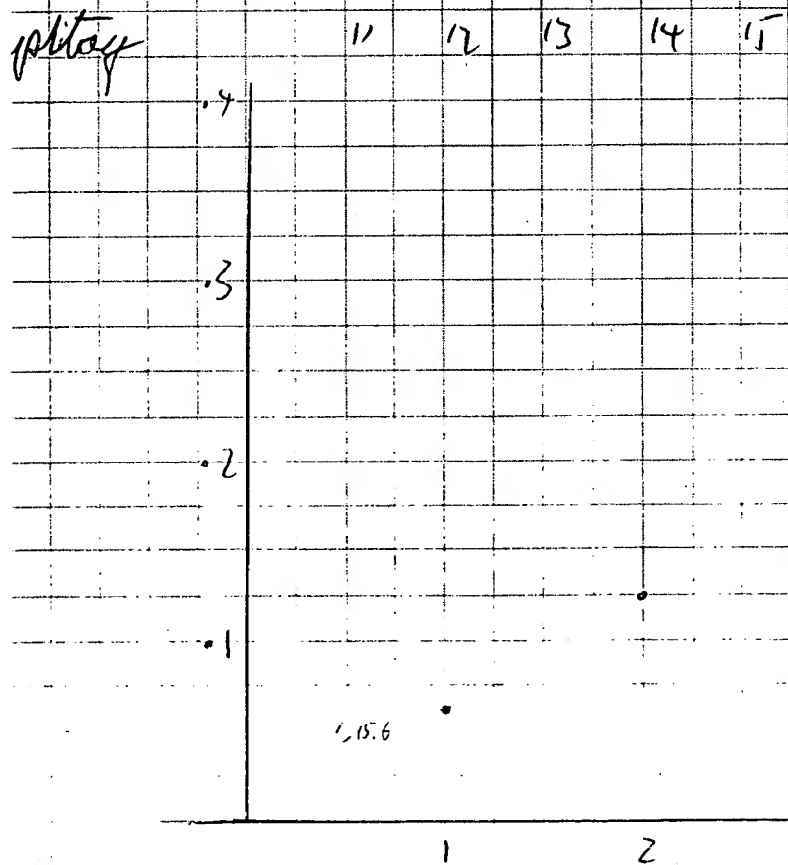
Solutions of Tag for PCR functional assay

Project N. _____ Exhibit L-85
Block No. _____ Appl. No. 09/558,421

11

Tube No.	#	1	2	3	4	5	1	2	3	4	5
CF u/ml		.0625	.125	.188	.25	.5					
lot#											
EM7414		5	5	5	5	5	3	3	3	3	4
g dilution buffer		395	195	129.3	95	45	237	117	77	57	27
VF =		400	200	133.3	100	50	240	120	80	60	36

R401 # 6 7 8 9 10



To Page No. _____

Read & Understood by me,

Polamp

Date

5/15/95

Invented by

Recorded by

Date

From Page No. _____

(P-3151) 3' end reaction can be Gf = 200 nM

33 mer correct (P13P, 9)	20 pmol/l (20 µM)	7.5	15 µl	✓	✓	~ 300 pmol 33 mer
32P dATP 10 mCi/µl		5	30 µl	✓		1.1 x 10 ⁶ cpm/µl
5-12-95		6	12 µl	✓		~ 900
5x Kinase buffer		1	3 µl	✓		
PNK 1 µl						
Hot						
		30	60 µl			
					37°C, 30'	
					55°C, 5'	

Plan for fidelity assay for pol ± 3' end

(33 correct P13P, 9)

(-) dCTP (+) dATG-TP

CCAGTG A A T T G A G T G G T A
 C T T A A G E T C G A G C C A T G G G C C C C

↑
 same 5' end as
 23 mer on mp19+

↑
 only 3 into
 for residue will
 have to do

↑
 most run times
 stopped here for
 quantitation
 at 49-51

↑
 n-1 is
 3 into downstream
 from primer
 3' end

Witnessed & Understood by me,

[Signature]

Date

5/15/95

Invented by

[Signature]

Recorded by

Date

5-9-95

To Page 1

3' exo in poly: TFi, Tne

From Page No.____

From Page No. — see P 136, 9 137, 9 for procedure: 200 nm primer (so its like a real PCR)

① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨ ⑩ ⑪


cocktail

10x PCR buffer
50 mM MgCl₂

5 _____ 2
1.5 ml _____ 2

60A ✓
18A ✓ (1.5mm)

34P β 3mer correct
p12 (5 μ m)
H₂O

2 

241 ✓
102 v_f - use 8.5 μl

TFI FrI 4-1395

10

TFI FrI'/PEI 4.13.51
(2.45u/l)

10

Tf1 in storage buffer
4.33 u/l (p8)

10

TF1 epimete WATF31010A-502
(3.5^u/d P.P)

12.4

rtag 5u/A 'EKBTI

10

The 3-245 1.72 u/l
(P. 13) dilute to 0.5 u/l

210

The 5-7-95 36^u/A

2	10	-
---	----	---

(according to Lig. F and see 1013
where I got $3(4\frac{1}{2})$).

where I got 54.2%
dilute to 0.5% Δ

1% Tween 20 / NP40

H.O.

Tag del beffer

$$v_f = 50 \lambda$$

74°C. remove 10 μ l to 5 μ l cryal seq stop
at 0.33, 1, 2 hr
run on 8% PAGE

see analysis of TFI / vent exp rate and TFI loss of full bag
33 may on p 46. To Page N

To Page N

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Date

Inv nted by

Date

Recorded by

PAGE 15 OF NOTEBOOK WAS BLANK

From Page No. _____

10 PCR buffer

50 mM MgCl₂

3 (JS) substrate

0.5 pmol / λ

EXT EFE 73

autoclaved, filtered H₂OMix A
110 μ l110 μ l44 μ l726 μ l \checkmark V_f = 990 μ lC_f = 1XC_f = 5 mM

(1 pmol / reaction)

acc
to
QC

1035

winds 0 2 4 6 8 10

tube # 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

4-30-95

TF1 (P. 8)
4.33 μ l / λ 0.46
0.92
1.39
1.85
2.3

Tf1 epicenter

EXT TF3 IN DA 502

3.5 μ l / λ (see P. 8)0.57
1.14
1.71
2.28
2.86

TF1 Fr I

4-13-95

dil none 1/100

1/10

1/100

H₂O 3 4.54 3.63 2.75 4.43 9.33 2.7 2.1

Tag storage buffer 2

Mix A 45 μ l

Tag dil buffer

V_f = 50 μ l5 3 \rightarrow

74°C, 60'

37°C, 60'

put tubes on ice

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J. Polanco

Date

5/15/95

Invented by

Record d by

Dat

5-12-95

To Page N

PET

Project N _____

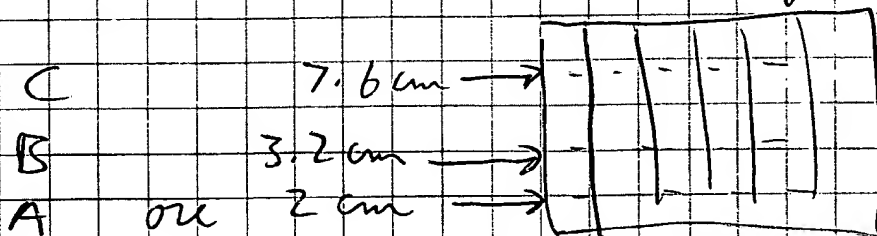
B k No. _____

17

g N — cut plate in half → get 20 x 10 cm plates

use 0.9 cm wide lanes
origin at 2 cm

save lanes
every 0.9 cm



spot 5 μ l, dry, spot another 5 μ l, dry
resolve in 2 N HCl

bring solvent front to top of plate

Dry by heat lamp, not more than 7 min

count bottom - 3.2 cm (= ori)

3.2 cm = 7.6

7.6 = top

for each

3 x 12 = 34 tubes

add 3.5 ml flour

count 34

To Page No. _____

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Polamp

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5-12-95

Project No. _____

Book No. _____

TITLE _____

From Page No. _____

CPM

no released

TFI 4.30-50	0	ori	1	4802.00
		main	2	673.00
			3	92.00
			4	4782.00
			5	709.00
			6	123.00
			7	4935.00
			8	702.00
			9	114.00
			10	4670.00
	2		11	743.00
			12	129.00
			13	4732.00
			14	669.00
			15	123.00
			16	4788.00
			17	767.00
			18	123.00
			19	4661.00
			20	613.00
TFI picenter	4		21	84.00
			22	4677.00
			23	636.00
			24	145.00
			25	4185.00
			26	767.00
			27	140.00
			28	4586.00
			29	774.00
			30	112.00
	6		31	4136.00
			32	887.00
			33	111.00
			34	4202.00
			35	793.00
			36	97.00
			37	1404.00
			38	396.00
			39	1801.00
			40	70.00
TFI FrI	0.02X		41	133.00
			42	2536.00
			43	27.00
			44	208.00
			45	2466.00

BKGD

31

0

22

0

37

0

31

0

31

0

53

0

48

0

20

0

19

0

5

0

1709

50%

2461

93%

2374

91%

Conclude no ds 3'epo activity is detected
in either LTI or Epicenter printed TFI pol

To Page No

Witnessed & Understood by me,

Date

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Date

5-12-95

Page N. _____

Epicenter TFI storage buffer

LTI & Tag storage buffer

Tris HCl

50 mM pH 7.6
(pH at Room Temp based on Tack call to Epicenter Tech line)

20 mM pH 8

need to increase molality from 2.0 mM to 5.0 mM and pH from 8 down to 7.0

NaCl

100 mM

0

need to add 100 mM NaCl to LTI SB

Glycerol

50 %

"

LTI & Tag as epicenter

DTT

1 mM

"

EDTA

0.1 mM

"

urea 20/NP40

0.5 % each

"

Experiment

1 M Tris HCl

pH (room Temp)

10 ml buffer G

0
5 μ
20 μ
20 μ
20 μ
20 μ
20 μ
20 μ

8.02 (expected 8.0)
7.99
7.92
7.81
7.77
7.72
7.67

add 125 μ l of 1 M Tris HCl to 10 ml TFI 4-30-95

W Cf = 20 mM Tris (in SB)
+ 12.5 mM Tris HCl added
32.5 mM

will follow this exact procedure for 10 ml of TFI 4-30-95
(pH 4.33 μ /l \Rightarrow new Cf = $\frac{10.0}{4.33} \times 4.33$
= 10.614 μ /l

add 182 μ l 1 M Tris pH 7.5 \Rightarrow Cf = 50 mM pH = 7.60 pH

Vf = 182 μ l + 125 μ l = 307 μ l
plus 307 μ l glycerol
Vtotal = 10.614 ml
plus 62 mg
mix end over end 30 to 60 min

NaCl 4 mM

Read & Understood by m , Bokun	Date 5/15/95	Inv nted by 	Date 5-12-95
		Recorded by	

Cf = 100 mM To Page No. _____

Stability study of A/in (no
TFI / vent mix. (5-16-88) ^{labeled}

From Page No.____

Follow p. 84, 9



Cheng et al

5x Cheng (no ATPs).
H₂O
activated DNA 3.7 mg/ml
dATG-C-TP 10 mM ea
32P dATP 10 mCi/ml
5-19.50 ref/date 3000 Ci/mmol

200 ml

637.9

135.1

5. ul

2' ml

glycerol 100 70 80

Time 10:00 PM

100 Ac.	0.5 m
100 MTD	2.0 m

mgO Ac	1.05
--------	------

0.5 mg/l

for 10 Rxns

$$V_f = 780 \mu l$$

① ② ③ ④ ⑤ ⑥ ⑦ ⑧ ⑨

A

981

TF1 LTISB 1.891 \longrightarrow
+ Vent (5-16.55)

TF1 Episcopus SB 2 \longrightarrow
+ Vant (5-16 ST)

TF1 Epicentre Enzyme + Vent 1.89 g/mol \rightarrow
(5-14-51)

$$V_f = 100 \mu l$$

680

remove 10 μ l \rightarrow 5 μ l 0.2m EDT

(spot 10 μ l on 6-FC) and remove 5 μ l to 5 μ l Kill solution
with cold DAPI (PP4, 9) and spot 2 μ l on PEI (PP4, 9)

at 0, 10, 20, 40 min

Repeated on p. 40

Conclusion: There is no real good way to do this experiment because Vent is present at very low level compared to ^{18}O incorporation is saturated at levels where turnover is barely detected by PEI method. Will have to settle for DNA 3' end QC assay for units \approx see p 26

To Page **M**

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Date _____

Inv nt d by

Date _____

✓ *Deer Leap*

6/9/95

Recorded by

5-11-55

*Turnover**Turnover*

ide No.

1247.00
3736.00
4880.00
6046.00
2170.00
3243.00
3897.00
5694.00
2774.00

Experiments 17

29 1925.00
30 3149.00
31 2983.00
32 3730.00
33 1091.00
34 1772.00
35 3000.00
36 3722.00

3405.00

4090.00

5613.00

3451.00

3498.00

5034.00

3820.00

2169.00

2604.00

2612.00

4580.00

2193.00

2755.00

5480.00

4542.00

2053.00

3113.00

2817.00

3182.00

*incorp**pmol*

1 68601.00
2 118176.00
3 155582.00
4 95344.00
5 125044.00
6 138326.00
7 93269.00
8 119376.00
9 167655.00
10 93777.00
11 116666.00
12 131003.00
13 109619.00
14 126936.00
15 143456.00
16 90599.00
17 103792.00
18 162204.00
19 96493.00
20 124924.00
21 162506.00
22 91166.00
23 125191.00
24 167630.00
25 84292.00
26 127063.00
27 153977.00
28 2135.00
29 975.00
30 110991.00
31 110539.00

1487
2562
5372

RKOD
RKOD

ave 110765 69.2 cm/pmol

To Page No. _____

sed & Understo d by me,

Edward Polap

Date

5/1/85

Invented by

[Signature]

Date

5-16-85

R corded by

From Page No. _____

X buffer "S"

1M Tris HCl 7.5 pH
0.5M EDTA
glycerol
3 me
3M KCl

20 ml ✓
0.2 ml ✓
80 ml ✓
0.357 ✓
16.7 ml ✓
1 L

cf
20 mM
0.1 mM
8%
5 mM
50 mM

passed 6 ml S200

wash equilibrated at 1 col vol/hr
(= 0.1 ml/min) for 2 hr

load 120 ml of Tne 36 u/l 5-7-95
(= 2% vol vol) (4320 units total)
by gravity.

elute at 1 col vol/hr
into 95 fr

collect 50 ul frns (30 sec/fr)
span ~0.5(??)
A

* with no detergent in buffer
maybe this is why activity died after
few weeks at 4°C (see P53)

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Date

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Date



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5-17-95

Project No. _____
Book No. _____

TITLE Assay 5200 fractions

24

From Page No. _____

Fr # 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25

Silicate 100 2 ul

Tio₂ 48 10 74

Results from
Top of P. 25 u/ml

Fr	mol	u/ml	Fr	mol	u/ml
10 1	9978.00	199	3 1	3216.00	0.97
13 2	9430.00		4 2	3990.00	1.2
16 3	16574.00		5 3	5279.00	1.6
19 4	12045.00		6 4	5148.00	1.55
22 5	10155.00		7 5	6128.00	1.8
25 6	7256.00	2.2	8 6	8950.00	2.7
28 7	6430.00	1.9	9 7	11386.00	3.4
30 8	6091.00	1.8	10 8	16350.00	4.9
35 9	3680.00	1.1	11 9	14464.00	4.5
40 10	2746.00	0.82	12 10	19127.00	5.8
45 11	2002.00	0.65	13 11	23242.00	7.0
50 12	1476.00	0.44	14 12	24609.00	7.4
55 13	1118.00	0.35	15 13	25276.00	7.6
60 14	694.00	0.2	16 14	24319.00	7.5
65 15	970.00		17 15	23374.00	7.0
70 16	603.00		18 16	16929.00	5.1
75 17	120535.00	75 cpm/mol	19 17	14764.00	4.4
			20 18	15075.00	4.5
			21 19	13028.00	3.9
			22 20	10781.00	3.2

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Date

Inv nt d by

Dat

To Pag 1

6/9/95

R c rd d by

5-17-15

g N	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30																																									
1/100	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30																																											
Results on P 24																																																																							
9-95	unit array on fr 13-17 : pool 20 µl of each																																																																						
B-17	1	2	3	4	5	6	7	8	9	10																																																													
100	2																																																																						
200	2																																																																						
400	2																																																																						
800	2																																																																						
1600	2																																																																						
3200	2																																																																						
5-7-95 3 u/l											11	13	1	163.00																																																									
1000											12	14	1	216.00																																																									
2000											13	15	1	115777.77	72%																																																								
4000																																																																							
8000																																																																							
pool	u/l																																																																						
3	1	16254.83	538	5.1																																																																			
4	1	12439.50	7.8																																																																				
5	1	5518.00	6.9																																																																				
6	1	2909.00	2.3																																																																				
7	1	1741.00	2.7																																																																				
8	1	866.00																																																																					
9	1	17122.00	355	53.2																																																																			
10	1	10433.3	45	6.5																																																																			
11	1	5683.00	70	7.0																																																																			
12	1	3137.00	47	7.7																																																																			

BI 625

2 > R x min

10, 74°C

will normalize 5200

pool 13-17 against 36% A

value for Tue 5-7-95

(see P 13) 00

pool 13-17 $\frac{7.7}{70.0} \times 36\%$

$= 3.9 \text{ u/l}$

$(3.9 \text{ u/l} \times 7 \text{ frms}) / (50 \text{ µl/frm})$

13.65 units recovered.

70.7 ave

applied only 36 (see P 13)

To Page No.

10, 74°C

will normalize 5200
 pool 13-17 against 36 u/l
 value for Tue 5-7-95
 (see P 13) 00

pool 13-17 (7.7 u/l) / (56 u/l)
 = 0.1375

= 3.9 u/l

(3.9 u/l) (7 frms) (15 µl/frm)
 13.65 units recovered.

70.7 ave.

applied only 3.6 (see P 13)

To Page No. _____

sed & Und rst od by me,

Polamp

Date

6/9/95

Invented by

Recorded by

Date

5-18-95
5-19-95

3' exp (QC) assay
for TFI / vent

From Page No.

(see P20 where turnover on PET didn't work)
follow assay on P 16-17

actual units of Vent
added based on 0.094 vent / 0.945
TA/v

Mix A P.16

4545

TFI/vent LTESB
(5-16-95)

~ 0.2

.02

.002

.0002

.00002

.000002

TFI/vent Epinephrine SB
(5-16-95)

TFI/vent with
epinephrine TFI (5-16-95)

H₂O

3

BKWD 68°C, 60 min
→ 88.00 21.31

BKWD start 2:35 pm

no dil 1 1684.00 4.87

1596

1/10 2 1323.00 5.49

1235

1/100 3 448.00 9.44

360

1/1000 4 143.00 16.71

55

1/10000 5 96.00 20.39

7

1/100000 6 101.00 19.92

13

Result: assay is not very h
in any range.
with assay at 1/10 and 1/100
in triplicate dilutions

To Page 1

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Date

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Date

Polansky

6/9/95

Record d by

5-22-95

Project No. _____

Book No. _____

TITLE _____

Results
2nd of enzyme dilution

28

From Page No. _____

Front middle ori	413.00	Sum 4406	amt of Triphates	To last from oil	Vend	282.00	Sum	amt
	443.00					681.00		
	3550.00					3216.00		
I SB	345.00	4050	314 244 520 199 3275 - 755	15% 15K00	BKG0	252.00	Sum	3193 on
	557.00					623.00		
	3148.00					3170.00		
I SB	185.00	3874	302 229 552 231 3434 - 596	15% 15K00	BKG0	85.00	Sum	an 7 32 41
	562.00					325.00		
	3127.00					3767.00		
I SB	308.00	4410	460 387 476 145 2995 - 1035	25% 25K00	BKG0	60.00	Sum	4669
	524.00					317.00		
	3578.00					4292.00		
I SB	272.00	4216	460 387 476 145 2995 - 1035	25% 25K00	BKG0	469.00	Sum	4669
	603.00					271.00		
	3341.00					4391.00		
I SB	325.00	4216	460 387 476 145 2995 - 1035	25% 25K00	BKG0		Sum	4669
	528.00							
	3383.00							
I SB	412.00	4216	460 387 476 145 2995 - 1035	25% 25K00	BKG0		Sum	4669
	509.00							
	2885.00							
I SB	453.00	4216	460 387 476 145 2995 - 1035	25% 25K00	BKG0		Sum	4669
	515.00							
	3004.00							
I SB	515.00	4216	460 387 476 145 2995 - 1035	25% 25K00	BKG0		Sum	4669
	434.00							
	3096.00							

With ss d & Und rst d by m ,

Polamp

Date

6/1/95

Invent d by

[Signature]

Dat

5-23-95

Rec rd d by

To Pag N

Result
5 λ enzyme dil

sg	N	TIME	total sum	% lost from front	Result															
1	front	324.00			<p>Result. The sum of all counts when enzyme is present (ave 2879) is less than for the no enzyme blank (ave 3713) so 22% of counts unaccounted for!</p>															
2	middle	726.00	1.00																	
3	ori	1780.00	1.00	2830																
4		348.00	1.00																	
5	ave	960.00	1.00	3117																
6		1809.00	1.00																	
7		458.00	1.00																	
8		830.00	1.00																	
9	1764	1703.00	1.00	2991																
10		483.00	1.00																	
11		608.00	1.00		<p>in each case ~ 1500 CPM is lost from origin (ie 3H removed by ep) but ~ 1/2 of that appears in front and middle. Also, why does any appear in middle it looks like 3H MP partly missing in middle since 3H runs in front, it may be quenched by broad contaminants that smear PEI at the front.</p>															
12		1726.00	1.00	2817																
13		477.00	1.00																	
14		515.00	1.00																	
15		1698.00	1.00	2690																
16		288.00	1.00																	
17		856.00	1.00																	
18	1658	1670.00	1.00																	
19		729.00	1.00																	
20		627.00	1.00																	
21		1452.00	1.00		<p>To label removed from dsDNA substrate</p> <table border="1"> <thead> <tr> <th></th> <th>2 λ enzyme mix</th> <th>5 λ enzyme mix</th> </tr> </thead> <tbody> <tr> <td>LTISB</td> <td>19%</td> <td>43%</td> </tr> <tr> <td>Epicate SB</td> <td>15</td> <td>46</td> </tr> <tr> <td>Epicate TFI</td> <td>26</td> <td>57</td> </tr> <tr> <td>Vert pol</td> <td>21</td> <td>50</td> </tr> </tbody> </table>		2 λ enzyme mix	5 λ enzyme mix	LTISB	19%	43%	Epicate SB	15	46	Epicate TFI	26	57	Vert pol	21	50
	2 λ enzyme mix	5 λ enzyme mix																		
LTISB	19%	43%																		
Epicate SB	15	46																		
Epicate TFI	26	57																		
Vert pol	21	50																		
22		632.00	1.00																	
23		511.00	1.00																	
24		1199.00	1.00																	
25		471.00	1.00																	
26		686.00	1.00																	
27	1354	1410.00	1.00																	
28		374.00	1.00																	
29		664.00	1.00																	
30		1398.00	1.00																	
31		200.00	1.00																	
32		786.00	1.00																	
33		1641.00	1.00																	
34		128.00	1.00																	
35		947.00	1.00																	
36	1573	1682.00	1.00																	
37		97.00	1.00																	
38		477.00	1.00																	
39		3007.00	1.00	3581																
40		66.00	1.00																	
41		591.00	1.00																	
42		3146.00	1.00	3803																
43		60.00	1.00																	
44		480.00	1.00																	
45	3122	3214.00	1.00	3754																

Project No. _____

Book No. _____

TITLE _____

30

From Page N. _____

Pool must assay for Nemo TFI/Vent m
same as P20 except used less units
10 per Tag unit assay P 12, 9

TFI Chemo unit assay m.

H ₂ O	1.056 ml	✓✓✓	35 Rows
5X Chemo buffer	350 μ	✓✓✓	
activated OVA 3.7 mg/ml	236 μ	✓✓✓	(0.5 mg/ml)
JATCG-TP 10 mM ea	35 μ	✓✓✓	200 μ M each)
3-Phid CTP 10 mM ea/ml	3.5 μ	✓	
	1.68 ml		use 48 μ l / Rxn

TFI Chemo unit assay m.

48 μ l \rightarrow

med TFI 4" ii #4 working stock

TFI 4.33 μ l / 1 (P.P)

1/250

2

2

1/500

2

2

1/1000

2

2

1/2000

2

2

TFI/Vent 5/16/5 LTISB

1/250

(TFI/Vent TFI/Vent)

Passing Epimut units

2

2

1/500

its ~4 μ l using

2

2

1/1000

my units (P.P) per TFI

2

2

1/2000

2

2

TFI/Vent 5/16/5 Epimut SB

1/250

2

2

1/500

2

2

1/1000

2

2

1/2000

2

2

TFI/Vent 5/16/5 (Epimut TFI)

1/250

1/500

1/1000

1/2000

74°C, 10'

dilute by adding 2.1 enzyme to 48 μ l Tag dil buffer for

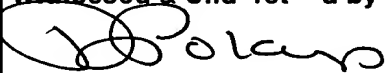
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Date

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Dat

T Pag 1



6/9/95

R c rded by

1-2455

ig No. _____

		SAM	CPM1	pmol	u/ml	
				(-BKG)		
				127	4.77	(was 4.33 on P8)
230 31 32 33	TFI	1	4165.00		5.14	
		2	2395.00		6.22	
		3	1575.00		6.9	
		4	1018.00		4.70	
		5	4108.00		6.7	
		6	3019.00		9.5	
		7	2232.00		7.7	
		8	1205.00		4.3	
	LTI SB	9	3788.00		5.0	
		10	2354.00		5.47	
		11	1425.00		7.62	
		12	1090.00		4.7	
		13	4135.00		5.0	
		14	2353.00		6.78	
		15	1688.00		7.07	
		16	1095.00		4.00	
	Epimeth SB	17	3543.00		5.06	
		18	2336.00		5.51	
		19	1431.00		7.13	
		20	1040.00		3.81	
		21	3388.00		4.64	
		22	2191.00		5.12	
		23	1354.00		5.31	
		24	857.00			
	Epimeth TFI	25	3746.00			
		26	2188.00			
		27	1548.00			
		28	866.00			
		29	4053.00			
		30	2474.00	71	5.3	
		31	1456.00			
		32	905.00			
	B/KGD Rx mix	33	322.00			
		34	72445.00	45.3 cpm/pmol		

will repeat this with 5 duplicates
of the 1350 µl dil for each
on P. 47

To Page No. _____

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Solano

Date

6/9/95

Invented by

Recorded by

Date

5-24-95

Project No. _____

Book No. _____

TITLE

units: 1.1X field test ("old")
new, BM 2X mix

34

From Page No. _____

Tag 1-31-95

Su/pl

1/125

1/250

1/500

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25

2

2

2

2

2

2

1.1X new (May 8, 1995)

no dil

1/2

1/4

2

2

2

2

2

2

1.1X old

no dil

1/2

1/4

2

2

2

2

2

2

BM 2X

1/2

1/4

1/8

2

2

2

2

2

2

-

Tag rxn mix
(P120, 9)48 ml →
1-0 ml

74°C, 10'

see cited # P35

for Tag 1/25 at 2 ml undiluted of sample
 new 6506 + 7080
 Tag 10692 + 12403 $(.04 \mu\text{g Tag by def}) = .023$

6729 + 6660

10692 + 12403

023

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Date

Invented by

Date

To Page 1

Polans

6/9/95

Rec rd d by

5-25-95

ag N _____

		pmol	u/μl			
1/125 1/25 1/125	1	10692.00	412	7.7	← (-333 BKGD)	7.7 is ~ right since this "5 u/l" is normalized to amplitude that was ~8 u/l
	2	5333.00		7.7		
	3	3112.00		8.0		
	4	12403.00		8.9		
	5	6387.00		9.2		
	6	3853.00		10.0		
1/2 1/4	7	6505.00	250	0.38 u/μl	Total units in in 50 μl at 1.1x μl	* in 50 μl at 1x
	8	4314.00		0.50	1.78 u	1.71
	9	2381.00		0.47	2.50	2.27
	10	7000.00		0.40	2.39	2.27
	11	4401.00		0.51	2.0	2.27
	12	2364.00		0.47	2.5	2.10
	13	6729.00		0.39	2.4	2.10
	14	3962.00		0.46	1.9	1.77
	15	2247.00		0.41	2.3	
	16	6660.00		0.38	2.2	1.73
	17	3659.00		0.38	1.90	
	18	1940.00		0.37	1.9	
1/2 1/4 1/8	19	3456.00		0.36		
	20	1705.00		0.32	0.77	
	21	1368.00		0.47	0.77	
	22	3028.00	1.7	0.55	0.84	
	23	2005.00		0.39	0.94	
	24	900.00		0.26		
25	333.00	BKGD				
26	62215.00	37.9 cpm/pmol				

~ red is average
total units in 50 μl
at 1x for the array of
undiluted μl of 1.1x mix
so use 1.76 units for "new"
and 1.75 units for old "field test"
as my first time points for these samples. (note expect ~2
old dilutions above indicate 2.22u and 1.89u for old and new)
see next array on P52 which is 1 month time point
using 2 μl undiluted

* mix is 1.1x
so at 1x mix is
(u/μl in 1.1x)

To Page No. _____

s d & Understood by me, Polansky	Date 6/9/95	Inv nted by 	Date 5-25-95
	Record d by		

36

Proj ct No. _____

Book No. _____

TITLE

unit array for stability
array of 1.1 x 10⁶ (p121, 9) (at
base of 1.1 x 10⁶ of 1.1 x 10⁶ 2.300 time from

From Page No. _____

1	8878.00	.036	98	
2	9039.00			
3	9623.00			
4	8220.00	.032	98	
5	8228.00			
6	8109.00			
7	8855.00	.034	106	
8	8307.00			
9	8584.00			
10	6857.00	.027	93	
11	7096.00			
12	6660.00			
13	9295.00	.035	105	
14	8535.00			
15	8519.00			
16	6780.00	.026	98	
17	5930.00			
18	5879.00			
19	8250.00			
20	8545.00	.034	104	
21	9288.00			
22	8605.00			
23	7590.00	.032	91	
24	7975.00			
25	7909.00			
26	7993.00	.031	99	
27	7301.00			
28	2863.00			
29	3151.00	.012		
30	3188.00			
31	7926.00			
32	7626.00			
33	7355.00			
34	8180.00			
35	8930.00	.034	100	
36	9000.00			
37	2662.00			
38	2885.00	.011	35	
39	2632.00			
40	8091.00			
41	7872.00	.031	89	
42	7722.00			
43	7664.00			
44	7828.00	.031	97	
45	8063.00			
46	10091.00			
47	9701.00			
48	10062.00			
49	10476.00			
50	10230.00			
51	101.00			
52	56411.00			
53	57488.00			

see you
result.
on p1

repeat in
section R
p153

will
(repeat)

10112 ave \Rightarrow 42 bpm \Rightarrow 7.99 units/s in
(expect 27 units 54/1 EKBT1
normalized to amplitude of 28

Witnessed & Understood by me,

Polamp

Date

6/9/95

Invented by

R. J. J.

Recorded by

Date

5-26-95
5-26-95

T Page No.

Stability of 1:1 X at room temp

ag N

0 time on P. 154, 9 3-13-55
 1 month P. 174, 9 4-11-55

assay same as P. 121, 9 for 4°C stability study
 used same assay mix as P. 5-25-52

amp slit #	Reaction tube #	ul enzyme 12x100	1% Tween 20 NP40	Tag mix assay mix (P. 121, 5)
	1-3	2		48 μ l
	4-6			
	7-9			
	10-12			
	13-15			
	16-18			
	19-21			
	22-24			
	25-27			
	28-30	↓	0.5%	
	31-34	3.64		
→ dil 1/2.5	34-36	2		
	37-39			
	40-42			
	43-45			
	46-48	47	0.5%	
	48-50	49		
5 μ l 1-31-55	52-56			
5 dil **	50-52			
	53 2 λ of "old mix"			
	54 2 λ of second mix			

74°C 10
 kill with 10 μ l
 0.5M EDTA
 spot 40 μ l
 on 6 FIC

10% TCA, 1% NaPP
 ↓
 3 x 5' in 5% TC
 1 x 5' in 95% EtO

12 μ l Tag dil buffer + 8 μ l sample #12
 * 5 λ tag + 620 λ dil buff

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		Recorded by	

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Book No. _____

TITLE _____

From I

	1	3864.00				
1	2	4350.00	.024	.037	2 months	1 month
	3	3113.00			65%	79
	4	155.00				
2	5	130.00	0.00090	0.033	2.7%	106
	6	133.00				
	7	4121.00				
3	8	4864.00	0.029	0.032	91%	106
	9	4225.00				
	10	3184.00				
4	11	3267.00	0.021	0.029	72%	93
	12	3240.00				
	13	3952.00				
5	14	3728.00	0.025	0.033	76%	91
	15	3817.00				
6	16	2585.00				
	17	2492.00	0.017	0.027	63%	78
	18	2795.00				
7	19	4009.00				
	20	4530.00	0.028	0.033	85%	94
	21	4334.00				
8	22	4199.00				
	23	4281.00	0.028	0.031	90%	98
	24	4326.00				
9	25	4248.00				
	26	3739.00	0.026	0.031	84%	90
	27	3922.00				
10	28	65.00				
	29	86.00	0.00053	0.022	0.24%	0
	30	93.00				
	31	4340.00				
11	32	3827.00	0.026	0.032	81%	98
	33	4038.00				
	34	5155.00				
12	35	5666.00	0.035	0.034	103%	—
	36	5301.00				
	37	3927.00				
13	38	3981.00	0.026	0.031	84%	97
	39	4352.00				
	40	4214.00				
14	41	3962.00	0.027	0.035	77%	93
	42	4287.00				
	43	3992.00				
15	44	3958.00	0.026	0.032	81%	106
	45	4278.00				
40°C	46	2014.00				
	47	2016.00	0.013			
40°C	48	1504.00				
	49	1509.00	0.0098			
40°C	50	5941.00				
40°C	51	6122.00				
40°C	52	6468.00				
40°C	53	49708.00				
40°C	54	40962.00				

(6.04 w/1) my definition

61.77

46819

same as P36 when corrected
for 30µl on GFC instead of 40µl
and 4 days decay of ²³⁰P

T Pag N

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Date

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Date

R cord d by

Page No. _____

sp act

$$\frac{\left(\frac{\mu\text{Ci}}{\mu\text{L}} \right) 46819 \text{ CPM}}{0.000 \text{ pmol } \gamma\text{-CTP}} (4) = 29.3 \text{ CPM/pmol(nt) DNA}$$

$$\left(\frac{6177 \text{ ave Tag CPM}}{29.3 \text{ CPM/pmol}} \right) \left(\frac{60 \lambda}{30 \lambda} \right) = 421 \text{ pmol DNA synthesis}$$

1 unit 10 nmol / 30' at 74°C

$$\left(\frac{.421 \text{ nmol}}{10 \text{ mol}} \right) \left(\frac{30'}{10'} \right) = 7.89 \mu\text{Ci}/\mu\text{L}$$

$$\frac{\mu\text{Ci}}{\mu\text{L}} \text{ of sample} = \left(\frac{.04 \mu\text{Ci}/\mu\text{L in Rxn}}{7.89 \mu\text{Ci}/\mu\text{L for Tag}} \right) \frac{\text{samples CPM}}{\text{Tag CPM}}$$

200 μM each dNTP

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6/9/95

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Date

5-30-95

Project No. _____

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TITLE _____

Repeat of P. 20 : stability of
TFI / Vent mixes

40

From Page No. _____

[A]

44.6.4 ✓
140 µl ✓
94.6 µl ✓
3.5 µl ✓
1.5 µl

Cf=

H₂O
5x Cheung buffer (no dNTPs)
activated OMA 3.7 mg/ml
ATG-C-TP 10 mM each

α^{32} P dATP 10 mCi/ml (ref 6-2-57)
3000 Ci/mmol

Tube # 1-4 5-8 9-12 13-16 17-20 21-24 25-28 29-32
(1) (2) (3) (4) (5) (6) (7) (8)

[A] 98 µl
2 µl TFI in epimut units
(its ~ 3.5 units/ml)
TFI LIESB 2
+ Vent (5-16-95) 10 µl →
0.09 units Vent/µl

2x 1x Cheung
← 0.18 units Vent in

TFI Epimut SB 2 →
+ Vent 5-16-95

TFI Epimut enzymed 2
+ Vent (5-16-95) 15 →
Vf ~ 100 µl

mistake: this is
0.09 units / 100 µl
be 0.18 - if this use
per 50 µl PCR

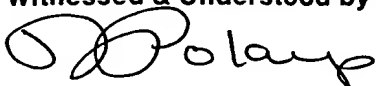
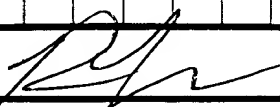
2 µl
0.045 µl
Vent diluted
in 1x Cheung
buffer

2 µl of Vent
2 µl of 2-
in 86.9 µl
1x Cheung

start with addition of enzyme to preheated mix.
remove 10 µl to 5 µl 0.2 M EDTA (spot 10 µl on GTC
and 5 µl to 5 µl Kellberg solution with cold dATP
(spot 2) on PET at 0, 5, 10, 15, 20 min.

resolve in 1 M LiCl

T Pag N

Witnessed & Understood by me, 	Date 6/9/95	Inv nted by 	Date 5-31-95
		Record d by	

g N .		cpm - background cpm ↑ specific activity		ave pmole turned over ave pmole incorporated ↑ should be constant		2 replicates of each time pt	
SAM		pmoles		% turnover			
CPM1		turnover					
1	5	885.00	ex $\frac{885-543}{103.31} = 3.31$	0.042		result: 1) turnover began to reach a plateau by 10 min. we expected turnover to continue increasing over time after DNA synthesis stopped. TFI competes w/ Vent at nick	
2	10	1241.00	4.76				
3	15	1074.00	5.14	0.069			
4	20	1269.00	7.03	0.065			
5		984.00	7.27				
6		1332.00	7.64	0.062			
7		1678.00	11.0				
8		1590.00	10.1				
9		830.00	2.78				
10		1213.00	6.49	0.021			
11		1195.00	6.31			2) turnover by Vent alone in Cheng buffer is lower than expected. 25% turnover was observed in another experiment. 453 pmole in 20 min. 25% 125 5min by Vent. The high signal to noise level for the Vent samples makes it difficult to say what the turnover is. Turnover is higher when Vent is mixed w/ TFI. TFI creates mismatches that are targets for Vent exo. do: repeat w/ 1, 2, 3, 4, 5 min time points and more Vent enzyme in the Vent alone samples, use new NEI plates	
12		1460.00	8.88	0.066			
13		555.00	0.116	0.055			
14		1228.00	6.63				
15		1225.00	6.60	0.062			
16		1425.00	8.54				
17		764.00	2.74				
18		977.00	4.20				
CPM3				0.039			
19		1212.00	6.48	0.028			
20		1453.00	8.81	0.043			
21		895.00	3.41				
22		772.00	2.22	0.061			
23		1009.00	4.51				
24		1365.00	7.96				
25		746.00	1.96	0.70			
26		438.00	1.02 X	0.47			
27		757.00	2.07				
28		609.00	0.64	0.14			
29		412.00					
30		578.00	543				
31		488.00	background				
32		693.00	no enzyme				

specific activity: cpm of 2nd spot of mix A 36438 $\bar{x} = 41,324$ cpm
2 replicates $\leftarrow + 40210$

$$\left(\frac{100 \mu\text{L rxn}}{2 \mu\text{L spot}} \right) \left(41324 \text{ cpm} \right) = 103.31 \frac{\text{cpm}}{\text{pmole (nt) DNA}}$$

$$\left(5000 \text{ pmole} \right) \left(4 \text{ bases} \right)$$

50 μM each dNTP in 100 μL rxn
50 $\mu\text{mole/L} \times 100 \times 10^{-6} \text{ L} = 0.005 \mu\text{mole} = 5 \text{ nmole} = 5000 \text{ pmole}$

To Page N . _____

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Invented by

Dat

Polamp

4/9/97

Recorded by

Cawley Combs

5-16-97

Project No. _____

Book No. _____

TITLE _____

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From Page No. _____

$$\frac{\text{sample cpm}}{\text{specific activity}} \times \frac{100 \mu\text{l rxn}}{10 \mu\text{l spot}} \times \frac{15}{10} \text{ dilution}$$

Incorporation

pmoles

1	49596.00	—	$\frac{49596}{203.31} \times 15 = 7,201$
2	74066.00	—	10,754
3	88521.00	—	12,853
4	95661.00	—	13,889
5	50395.00	—	7,317
6	69543.00	—	10,097
7	82738.00	—	12,013
8	93515.00	—	13,578
9	45114.00	—	6,550
10	64768.00	—	9,404
11	81250.00	—	11,797
12	96711.00	—	14,042
13	49095.00	—	7,128
14	71796.00	—	10,424
15	81335.00	—	11,809
16	95798.00	—	13,909
17	50290.00	—	7,302
18	70938.00	—	10,230
19	88754.00	—	12,887
20	98147.00	—	14,250
21	48881.00	—	7,097
22	85245.00	—	12,377
23	85694.00	—	12,442
24	91420.00	—	13,274
25	1932.00	—	281
26	2581.00	—	375
27	3000.00	—	436
28	3120.00	—	453
29	854.00	—	123
30	777.00	—	113
31	31183.00	—	26.6
32	32487.00	—	70.7
33	9.00	—	
34	6.00	—	

2ul mix A = 36438.00 - \bar{x} 41,324 cpm
 46210.00 for calculation of
 specific activity

105 cpm/pmol

Synthesis ~~test~~ was almost complete by 10 min.
 By 20 min. ~14 nmoles of the 20 nmoles
 had been incorporated - hi, in the 1st

To Page N

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Date

Invented by

Date

Recorded by

new dilution of rlog wt EKBT1
to 5^u/ml

Project No. _____

Exhibit L-100

Book No. _____

Appl. No. 09/558,421

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EKBT1
323 u/ml (see p91, 9)

157.2 ml

Tag storage buffer
(Princl detergent)

10 ml
 $V_f = 10^{.157}$ ml

(cf = 5^u/λ)

mix end over end 1 hour

storage buffer is from 12-7-94
with Princel Detergents

To Page No. _____

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Polansky

Date

6/9/95

Invented by

Recorded by

Date

5-31-95

Project N

B ok No.

TITLE

primer degradation using TFI//
(can see P 14, reaction # 11)

44

From Page N

(1) (2) (3) (4) (5) (6)

5 X Chemg
(no dNTPs)20 μ l —————→320 "33 mer correct"
same as (P 12 and 14)

4 —————→

5 μ MTFI/Vent 5-12-95
CTI SB

10

TFI/Vent 5-13-95
Epiarch SB

10

(0.9 units vent
in 10 μ l res)TFI/Vent 5-14-95
Epiarch TFI

10

TFI LTI 4.33 μ l (P.8)

10

Vent .09 μ l

10

Lot #17 (opened 2-24-95)

LTI SB P.6 (same stock as in TFI P.8)

10

H₂O

66

V_f = 100 μ l* 20 μ l Vent.
diluted into
CTI Tag SB
2 μ l Vent
42.4 μ l Tag SB
44.4 μ l

68°C

remove 10 μ l to 5 μ l appt res

stop vol at 0, 5, 10, 20, 40, 60, 80, 100 m

add enzyme on ice → take 0 time point,
start timing when thin walled tube
put in prewarmed 9600 at 68°C

1.6 % PAGE

~ 44 watts (Volts range from 1600
get ~ 12 cm/hr for BPB

To Page N

Witnessed & Understood by m

Date

Investigated by

Date

Recorded by

Polansky

6/9/95

6-2-95

check 5'P 33mer (P44) on P61

Project No. _____

Block No. _____

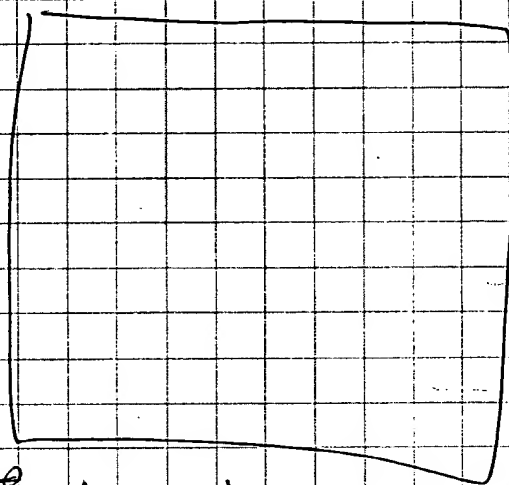
45

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3'P-33mer is $\sim 10 \times 10^6$ cpm/ μ l
↓ dilute $1/1000$

1.5×10^{-6} M
~~65 mmol nt~~ of 23mer \sim 20 ~~mm~~ nt
5 μ l H₂O
5 μ l

10 mM dATP stock



RT 2.0 min

RT 25.0 min

2.0

25.0

100 mM stock
→ 1:5 dilution
10 μ l stock, 40 μ l H₂O
TE

1 M ATP
1 M dATP

1 M 23mer
cold

33mer
1/1000

1 2 3
2 2 2
2

To Page No. _____

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Date

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Recorded by

Date

6-2-95

Jojoalays

From Page No. _____

Result:

1. ice does not shut down 3' exp. start with Mg. rept (?)
2. there is very rapid loss of first 1-10 nts, then very slow degradation. is next time, need 'less engaged' and/or shorter time points
3. No apparent ends of 5' exp. activity for TFI alone as seen on P14. Here its 5' TFI (4.33 u/l) per 5' compared to 2 u/l on P14 but still should see plenty of loss of full length here based on rate seen on P14. also was P14 but on P14 try ± JNTPs also P14 has JNTPs present but not here. Maybe some kind of primer extension involved in loss of full length primers - is extension to many long contributes to apparent loss of primer or to production of a 5' exp. target - could be primer primer extension or hairpin within 33 mer.

Witnessed and Understood by me,

D. Polay

Dat

6/9/95

Invent d by

Rec rd d by

Dat

6-2-95

To Page No.

Primer degradation by TFI/Vent

Project N _____

Exhibit L-102

Bo k N _____

Appl. No. 09/558,421

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'95-6/6/95

purpose: To measure 3'→5' exonuclease activity of TFI/Vent using the primer degradation assay.

background: An earlier trial of this experiment (NB 10 page) was done by removing aliquots of the rxn at time points 0, 5, 10, 20, 40, 60, 80, 100 min. The primer was degraded almost to the maximum amount by 10 min. Since we want to determine the 3'→5' exo activity rate, we need to find the linear range of the assay. This can be done by taking shorter time points or by taking a single time point on a series of enzyme dilutions (doubling [enzyme] should double extent of degradation in the linear range of the assay). We'll do this trial expt w/ just 1 enzyme sample - TFI/Vent in LTI SB - and 13 different dilutions. Once the linear range is found, we can repeat the exp. just on that range.

materials: ^{32}P & ATP for end labeling primer
primer = 33mer correct
Taq dilution buffer - cc aliquot
LTI storage buffer - RL aliquot
TFI/Vent enzyme mix - from stability study
9600 PCR machine & tubes
5x Cheng buffer - cc aliquot
PNK = T4 kinase & buffer - ~~SEL~~ LTI - new
8% sequencing gel & buffer - LTI premade
① stop buffer
sterile H_2O

To Page No. _____

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6/9/95

Invented by

Recorded by

C. J. Conk

Date

6/8/95

From Page No. _____

Procedure

4/5 ① end-label the 33-mer primer w/ ^{32}P & ATP = Kinase reaction
reference = notebook 10 p 12

mix:	H ₂ O sterile	25ul	✓	+ 25ul	added after 1st 5' con. were added
	5x Kinase buffer	12ul	✓	+ 12ul	
20.0ul	33mer constant	15ul	✓	+ 15ul	
AC4521	^{32}P & ATP 10mCi/mol	5ul	✓	+ 5ul	
	PNK 1u/ul new FES419	3ul	✓	+ 3ul	
		60ul		120ul	

incubate 37°C, 30min ✓
~55°C, 5min ✓

store labeled DNA + unincorporated label at -20°C - run some ok

4/5 ② make a ~~8~~ 8% denaturing-sequencing gel + buffer → both premade by

- 1, 75mL bottle of 8% mix (cold room) + 450ul ^{10}B AP (made fresh)

0.0868g AP

363ul H₂O → 0.0868g
0.868mL

- after pouring gel, shake up remaining gel mix so it can be used to fill leaks, ect.

- store gel upright, ON, at RT w/ H₂O-soaked towels and saran ✓

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Rawlyn C. Smith

Date

6/9/95

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Project No. _____

Book No. _____ TITLE _____

4

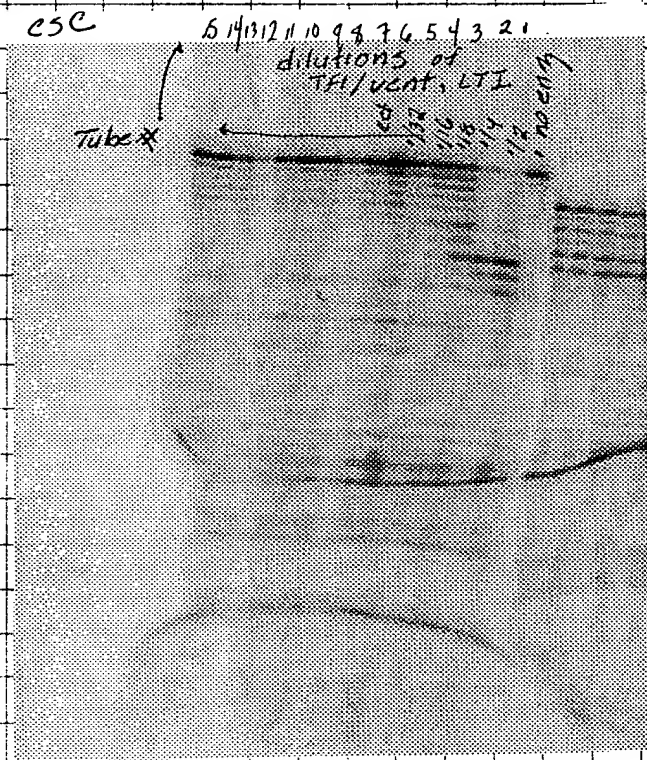
From Page No. _____

- B9D ran down to bottom glass clamp on gel rig, ~1hr, ^{constant} 1700V, ~6.5W
- transfer to ^{drain buffer} Whatman paper
- cover w/ saran & cut to size
- dry - 2 pieces whatman under gel, saran over gel, dry ice in trap
- set vacuum & heat for 1hr = 12:45-1:45 PM
- set in phosphorimager cassette - bottom to bottom w/ saran ~2 PM for ON exposure

Result: ON exposure on phosphorimager

csc

C:\DATA\CC.GEL 1995:06:07 07:57:48, Range = 0.11-10000.00 Counts, 0.50x



Conclusion: The 1/4, 1/8, 1/16 dilu
gene span the linear range
the primer degradation
Now, we'll do a cou
time pts of each
dilution to gather
better ^{data} from the lin
range. The data w/
used to show ^{3-5'} exo ac
for stability study.

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Paula Comb

Page N — 6/6/95

Purpose: To determine the relative mobility of the ^{32}P -33mer correct primer on a PEI plate, developed in LiCl.

Background: Originally we wanted to determine the specific activity of the ^{32}P -33mer primer that was used in the primer degradation assay w/ TFI/Vent (NB 10 p). However, we later decided that it is not important to find the specific activity since we can do a no enzy. control each time the assay is done. Now we want to determine the mobility because we observed that the cold oligo did not run as expected on the PEI plate, and we just are curious about how where the oligo ran.

Materials: cold 33-mer correct
 ^{32}P 33mer correct - labeled on 6/9/95

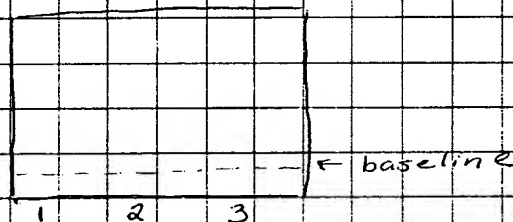
ATP

PEI plate

1M LiCl

scint vials = cocktail

Procedure: > spot on PEI plate →



20mM ATP 2ul

2ul

20mM 33mer

2ul 2ul

 ^{32}P 33mer

2ul

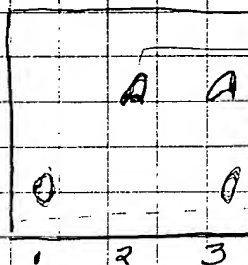
> set plate on developing chamber w/ 1M LiCl ~1hr ran 1/2 way

> circle control spots (ATP + cold 33mer) in lane 3 under UV light → sketch of how plate looked

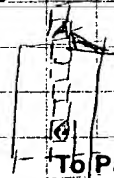
8 ATP ^{32}P
 ADP
 ^{32}P -33mer*
 cold 33mer
 PP. ^{32}P *

cut

> lane 3 into 8 pieces and count in scintillation counter



expected to stay at base, but ran near the moving front



To Page No. _____

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Polansky

Date

6/9/95

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6-12-95
 Recorded by
 Carolyn P. Smith

Date

6/9/95

Project No. _____

Book No. _____

TITLE _____

From Page No. _____

CSC

Result:

PAGE:

USER: 1 ID:32P 1.0 CPM PRESET TIME: 1.00 TUE 06 JUN 1995 15:36
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N RS232:N
 H#: 0 ADC:N BCF:N RCM:N
 CHANNEL 1-LL: 0 UL:1000 2SIGMA: 0.05 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR:
 DATA CALC: CPM. UNKNOWN REPLICATES: 1 NORM FACTOR: 1.00000
 HALF LIFE(DAYS):N

SAM	CPM1	TIME	EF
1	111.00 - baseline	1.00	
2	307.00 - area around where cold ATP	1.00	
3	136.00 standard ran	1.00	
4	117.00	1.00	
5	60.00	1.00	
6	215.00 - area around where cold 33mer primer ran	1.00	⇒ primer runs like dAMP
7	27.00 - near solvent front	1.00	

The labeled 33-mer primer ran like the dAMP runs on a PEI plate, up near the solvent front, not at the origin

This result was expected from the information we heard from the chemistry group: DNA stays at the origin because of its large size

oligonucleotides run like dAMP because they have same charge:mass ratio as dAMP

To Page 1

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Paulen P. P. P.

TfI/Vent primer degradation assay - time course on serial dilutions Book No. _____ for a time point of stability study

ig N - 5
 purpose: To measure 3'-5' exonuclease activity of TfI/Vent mixes in 3 buffers at the zero time point of stability study

background: Linear range of assay determined in previous expt, NB11 page 1 - dilutions 1/4, 1/8, 1/16 looked good
 - linear range $\hookrightarrow 0.045$ units

- now do time course of these dilutions
 1/4 dilution $\rightarrow \frac{0.09 \mu\text{L}}{4} \times 2 \mu\text{L} = 0.045$ units 1/8 dil $\rightarrow 0.0225$ units 1/16 $\rightarrow 0.0113$ units in 10ul reactions

materials: ^{32}P 33mer correct - labeled on 6/5/95

TfI/Vent in LTI, epicenter SB, epicenter TfI

8% gel
 mix A

* Vent dilution 2000 u/mL
 2ul vent Lot 17 2/24/95
 44.4ul TAE 513
 44.4ul at 0.09 u/uL
 22.2x dil

procedure:

1) make mix A, enough for 14 rxns - 90ul per rxn
 per rxn $5.6 \mu\text{L} \times 14 = 78.4 \mu\text{L}$ $20 \mu\text{L} \times 14 = 280 \mu\text{L}$ 5x Cheng
 $4 \mu\text{L} \times 14 = 56 \mu\text{L}$ 32P 33mer sum stock
 $66 \mu\text{L} \times 14 = 924 \mu\text{L}$ $990 \mu\text{L}$ H₂O
 $90 \times 14 = 1260 \mu\text{L}$ mix A

= TfI/Vent in LTI SB
 = " in epicenter SB
 = " in epicenter TfI

= vent*

2) each enzyme/buffer mix

stop tubes	37-40	37-40	37-40	37-40
15	25-28	29-32	33-36	37-40
13-16	17-20	21-24	25-28	29-32
1-4	5-8	9-12	13-16	17-20

mix A 90ul 90ul 90ul
 H₂O 8 6 4
 undiluted enzyme (0.09 u/uL) 2 4 6

take 3, 6, 9, 12 min time points by removing 10ul of rxn to 5 ul stop in small tubes, keep on ice

start rxns 1 min apart time on clock - 0 1 2 start

3	4	5	stop
6	7	8	stop
9	10	11	stop
12	13	14	stop

To Page No. _____

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TITLE _____

10

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Note: samples Vent/TfI in LTI, 3 min 2nd > only 5th of can was stopped - load more 4.5 instead
3 min 4th

4:50 PM - ~6:15 PM 1700V constant, -4.5W, gel was dried & put in P.I.
 * 39-46 may be underloaded due to problem expelling full vol. from
 order: control, 1-48 where 1-12 are TfI/Vent in LTI SB
of samples on gel 13-24 are TfI/Vent in epicenter SB
 25-36 are TfI/Vent in epicenter TfI
 37-48 are Vent alone.

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TITLE

Repeat unit assay for TF1/V.

48

From Page No. _____

Wms TF1/Vent mps of A J-
with 5 duplicate dilutions
for optimized signal/noise

Use 2 μ l of 1/250 dil for
and linearity vs (units)

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

TF1 Check unit
assaying (same
as P. 30)

47 —————→

TF1 4.33 μ l (P. 8)
1/250 dil *

2 —————→

TF1/Vent LTISB

5-16-95 (Nin Guanid)
1/250

2 —————→

TF1/Vent Epicent SB

5-16-95 1/250 dil

2 —————→

TF1/Vent (Epicent TF1)

5-16-95 1/250 dil

2 —————→

VF-50 μ l

74°C, 10' → 10 μ l 0.5M EDTA → spot 40 μ l on

* all dilutions are done as 5 separate dilutions
of 2 μ l Enzyme into 498 μ l Tag dilution buffer

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1	4163.00
2	4429.00
3	4636.00
4	4646.00
5	4349.00
6	4550.00
7	4529.00
8	4623.00
9	4350.00
10	4315.00
11	3995.00
12	4339.00
13	3732.00
14	4695.00
15	4428.00
16	3975.00
17	4584.00
18	4541.00
19	4297.00
20	4412.00
21	259.00
22	84613.00
23	87557.00

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James A. [Signature]

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6/19/95

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6-9-95

Test run of PEI plates - prior to turnover exp

ag N. _____

2/95

Purpose: To test how well a fresher batch of PEI plates can resolve dAMP from dATP and how tight/clean the spots are. This is being done prior to using this batch of plates for another TFI/Vent turnover experiment

Background: Last time the dAMP spot did not resolve well from the yellow "junk" that runs near the 1M LiCl solvent front, making it difficult to cut out + count just the dAMP (without Pi) for accurate turnover results. We'll try washing a different batch of plates - from Jesse - in dH₂O + drying them 1st, before running samples and compare to unwashed plate. Also we see if running a whole plate gives better resolution than a 1/2 plate.

materials: PEI plates - from Jesse ← Macherey Nagel Polygram cell 30
 Kill soln = ~20mM dATP
 20mM dADP
 20mM dAMP
 100mM EDTA
 1M LiCl - fresh, see recipe on next page
 PEI/UV
 Aldrich cat # 212,288-2

To Page No. _____

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Date _____

6/12/95

Carolyn E. Smith

see P14 apparent exo present
in PCR buffer Project No. _____
50 P44, no exo in Book No. _____
From Page No. _____

Repeat apparent exo result for TFI
on P14 with different primer
~~to home + hand check~~

Exhibit L-107
Appl. No. 09/558,421

① ② ③ ④ ⑤ ⑥ ⑦ ⑧

10x PCR buffer
50 mM MgCl₂

5 —————→
10.5 μl —————→

5 ✓
15 ✓

5x Cheung (no dUTPs)
(see P200)

10 μl —————→ 10 ✓

32P 33mer correct (P11) 5 μm
34P 23mer "AC" 5 μm
32P 42mer "fidel" 5 μm
see P12, 14 for method

2 2 2 2 ✓
2 2 ✓
2 2 ✓

Tag storage buffer

10 10

TFI 4.33 μl (P.8)

X10 —————→

H₂O

31.5 —————→ 28 —————→ 31.5 28 ✓
V_P = 50 μl

74°C remove 10 μl to 5 μl cycle seq stop sol
at 15 min 60 min

run on 8% PAGE

* Zero time point:

1. mix buffer^{mix}, 3²P primer (MgCl₂ if needed) and H₂O. Volume =
2. remove 8 μl to 2 μl Tag storage buffer and 5 μl cycle seq. for 0 (no enzyme) time point.
3. now have 32 μl of reaction^{mix} left preheat to 74°C, add 8 μl TFI so V_P = 40 μl again and remove 10 at 15 and 60 min to 5 μl cycle seq stop sol

T Page 1

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Date

Demetrius Polaris

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6-13-95

32P

oligos (follow P12, 14 to 5 μ M)

Proj ct N _____

Book N _____

51

Pag No. _____

5X kinase buffer
"correct" 20 μ M
1379

[1]

[2]

[3]

4 μ l

5 μ l

✓

✓

mer AC(P1365)
100 μ M

1 μ l

✓

← 23mer has termin
A instead of G
its called "AC"

mer "fidel" 6-13-95 old Temp
100 μ M
ATP 1 μ M (ref 6-16-95)
VR
H₂O

This
is different from
Fidel Temp P54

1 μ l

✓

2 μ l

✓

← (5X less ATP
than on P12)

1 μ l

✓

8

12

12

✓

$V_F = 20 \mu$ l

37°C, 30', 5', 55°C

2 mer fidel

51351 CAC (012)

56.89 nmoles primer
56.89 μ l H₂O

F=100 μ M

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From Page No. _____

procedure: - deliver 10ul of kill soln to 1.5ml Cppendorfs x 1-
- label tops 10' 20' 30' 40' 50' 60', set on ice until

rxn #

1

2

3

4

stop tube #

1-~~6~~7-~~12~~13-~~18~~19-~~24~~

mix A

98ul / rxn

prewarm to 68°C

0.15 u/l Vent

2ul

start rxns by adding eng., keep at 68°C in 9600

Lot # 17

opened 2-24-95

0.10 u/l Vent

0.05 u/l Vent

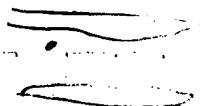
no eng

2ul x change
LTI SB
= Tag SB

100ul

- remove 10ul rxn to stop tubes at each time pt (10', 20', 40', 50')
- spot 10ul / GFC + 2ul PEI
- 10ul of mix A x 3 (for determination of specific activity)

Test of old (Baker) PEI plates - 2ul, cold kill soln spotted



- old plate -> dAMP runs w/
solvent front junk

- new plate -> dAMP runs ~ 1/2
between solvent front & or

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Pawlin P. mbs

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6/13/95

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Incorporation (pmoles)

20

From Page No. _____

(cpm specific activity) (100ul rxn / 10ul spot) (20ul / 10ul)

0.3 mits

1.2 units

0.1 units

no eng.

55 21752.00 - 936 pmoles
56 40494.00 - 1742
57 50701.00 - 2745 2181
58 63810.00 - 2723 2745
59 63423.00 - 2723
60 61923.00 - 2663

61 12710.00 - 547
62 21727.00 - 934
63 32040.00 - 1378
64 39939.00 - 1718
65 43064.00 - 1852
66 51401.00 - 2211

67 9060.00 - 390
68 14810.00 - 637
69 19948.00 - 858
70 24421.00 - 1050
71 31940.00 - 1374
72 30490.00 - 1311

73 420.00
74 540.00
75 299.00 $\bar{x} = 348$
76 310.00 $n = 6$
77 197.00
78 323.00

79 923719.00 10ul spot of mix A
80 973931.00 $\bar{x} = 929,40.2$ for specific activity
81 890737.00

The ^{observed} specific activity of mix A is 2x higher than anticipated by the following calculation:

$$\frac{10 \mu\text{Li}}{\mu\text{L}} \times \frac{2.2 \times 10^6 \text{ cpm}}{\mu\text{Li}} = \frac{2.2 \times 10^7 \text{ cpm}}{\mu\text{L}} \times \frac{3.4 \mu\text{L in mix A}}{1568 \mu\text{L A}} = \frac{8.7 \times 10^7 \text{ cpm}}{1568 \mu\text{L A}} = 5.6 \times 10^4 \text{ cpm}$$

0.925 2 days to ref. date

$$\frac{5.6 \times 10^4 \text{ cpm}}{\mu\text{L A}} \times 10 \mu\text{L A spotted} = 5.6 \times 10^5 \text{ cpm expected}$$

9 x 10⁶ cpm
9.3 x 10⁵ cpm observed

I don't know where the error came from, but the results should still be consistent within this experiment

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6/14/95

TfI/Vent in LTISB: turnover and incorporation
5 replicates of 3 time points

Book No. _____

Fig N. _____

purpose: To ^{more} accurately determine turnover by TfI/Vent in LTISB.
- 5 replicates of the 5min and 10min ^{15min} time points, which are within linear range of the assay. This data will be used for the stability study.

ckground: also see NB10 page for a time course of turnover, 2 replicates

materials: new PET plates,
mix A from 6/13/95
TfI/Vent in LTISB
Kill soln from NB11 page 14 = 20mM each dA-MDP-P
100mM EDTA

procedure: - deliver 20ul Kill soln to 1.5ml stop tubes 1-18 ✓

rxn x 5	1	2	3	4	5	6
top tubes	1-3	4-6	7-9	10-12	13-15	16-18

mix A 98ul ————— prewarm to 68°C —————>

TfI/Vent mix 2ul - add enzyme to start rxns
LTISB 2ul

wick plate

incubate at 68°C

100ul

> At 5, 10, 15 min remove 20ul rxn to the 20ul Kill soln in stop tubes, mix well & keep on ice

> spot 10ul/GEC filter (1-15) spot rxn 6, no eng control 4x per time pt
2ul/PEI plate (1-15) 27 x 2 = 54 = 12 spots

2ul
no enzyme control
2ul LTISB = Tag SB

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6/14/95

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6/14/95

22 Results:

Project No. _____

Book No. _____

TITLE _____

SAM	CPM1
odd = dAMP	
even = dADP	
1 > 5	950.00
2	72035.00
3 >	1727.00
4	71656.00
5 > 5	2488.00
6	67554.00

replicate 2	7 > 5	1176.00	- 163
	8	72213.00	
	9 > 10	1768.00	- 304
	10	69650.00	
	11 > 15	2189.00	- 404
	12	69544.00	

replicate 3	13 > 5	1219.00	- 173
	14	71519.00	
	15 > 10	1784.00	- 308
	16	70384.00	
	17 > 15	2314.00	- 434
	18	69324.00	

replicate 4	19	1189.00	- 166
	20	71061.00	
	21	1914.00	- 339
	22	72483.00	
	23	2096.00	- 382
	24	67757.00	

replicate 5	25	1032.00	- 128
	26	70489.00	
	27	1714.00	- 291
	28	64939.00	
	29	2289.00	- 428
	30	64174.00	

4 spots	31	612.00	
no ena	32	75976.00	
	33	494.00	
	34	79452.00	
	35	421.00	
	36	79068.00	
	37	587.00	
	38	78840.00	

4 spots	39	460.00	
no ena	40	80087.00	
	41	481.00	
	42	78697.00	
	43	419.00	
	44	78893.00	
	45	404.00	
	46	76101.00	
	47	450.00	
	48	68672.00	
	49	549.00	
	50	67776.00	
	51	498.00	
	52	73381.00	
	53	559.00	
	54	72680.00	

4 spots	55	620.00	spot near dAMP
no ena	56	1714.00	spot near dADP

4 spots	57	620.00	spot near dAMP
no ena	58	1714.00	spot near dADP

4 spots	59	620.00	spot near dAMP
no ena	60	1714.00	spot near dADP

4 spots	61	620.00	spot near dAMP
no ena	62	1714.00	spot near dADP

4 spots	63	620.00	spot near dAMP
no ena	64	1714.00	spot near dADP

4 spots	65	620.00	spot near dAMP
no ena	66	1714.00	spot near dADP

Turnover (cpm)
$$\left(\frac{\text{cpm} - \text{background cpm}}{\text{specific activity}} \right) \left(\frac{40}{200} \right) \left(\frac{100}{2} \right)$$

$$\left(\frac{950 - 495}{419} \right) \left(\frac{40}{200} \right) \left(\frac{100}{2} \right) = 109$$

$$163 \quad 294$$

$$476$$

$$\bar{x} \text{ Turnover} \pm 1SD$$

$$n=5$$

$$5' \Rightarrow 148 \pm 28$$

$$10' \Rightarrow 307 \pm 19$$

$$15' \Rightarrow 425 \pm 35$$

$$70 \text{ Turnover}$$

$$100 \times (\text{pmole dAMP} / \text{incorporation})$$

$$3.3\%$$

$$5.0\%$$

$$5.5\%$$

background for turnover

$$\bar{x} = 495 \pm 69 \text{ cpm} = 118 \text{ pmole/s} \rightarrow \text{samples are only 1 to 3 above background}$$

$$n = 12$$

cc 6/14/95

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Deena Pokup

Date 6/19/95

Investigated by _____

Recorded by _____

Date 6-19-95

cc 6/14/95

Date

cc 6/14/95

age N _____ Incorporation (pmol)
 (cpm/specific activity) $\left(\frac{100\mu\text{L rxn}}{10\mu\text{L spot}}\right) \left(\frac{40\mu\text{L}}{20\mu\text{L}}\right)$

5' 93072.00 93072/419 $\left(\frac{100}{10}\right) \left(\frac{40}{20}\right) = 4443$

10' 107957.00 - 5153

15 140583.00 - 6710

5' 107888.00 - 5150

10' 116159.00 - 5545

15 157153.00 - 7501

5' 89224.00 - 4259

10' 129878.00 - 6199

15 158185.00 - 7551

5' 86678.00 - 4137

10' 129770.00 - 6194

15 146342.00 - 6985

5' 71757.00 - 3425

10' 127388.00 - 6081

15 158825.00 - 7581

no eng 285.00
 5 355.00
 no eng 291.00
 10 300.00
 no eng 310.00
 15 262.00

background

$\bar{x} = 301 \pm 31 \text{ cpm}$

$\approx 14.4 \text{ pmol/L}$

$n = 6$

839570.00 100L mix A

831885.00 spotted 3X

840299.00 $\bar{x} = 837,251$

$\bar{x} \pm 1SD$ Incorporation

$n = 5$

5' $\Rightarrow 4283 \pm 620 \text{ pmol}$

10' $\Rightarrow 5834 \pm 467 \text{ pmol}$

15' $\Rightarrow 7266 \pm 395 \text{ pmol}$

specific activity = $837,251 \text{ cpm} \times \frac{100\mu\text{L rxn}}{10\mu\text{L spot}} = 419 \text{ cpm}$
 $(5000 \text{ pmol}) 4 \text{ pmole (nt)}$

To Page No. _____

is d & Understood by me,

Research Polansky

Date

6/19/95

Invented by

Record d by

Carlyle Conk

Date

6/14/95

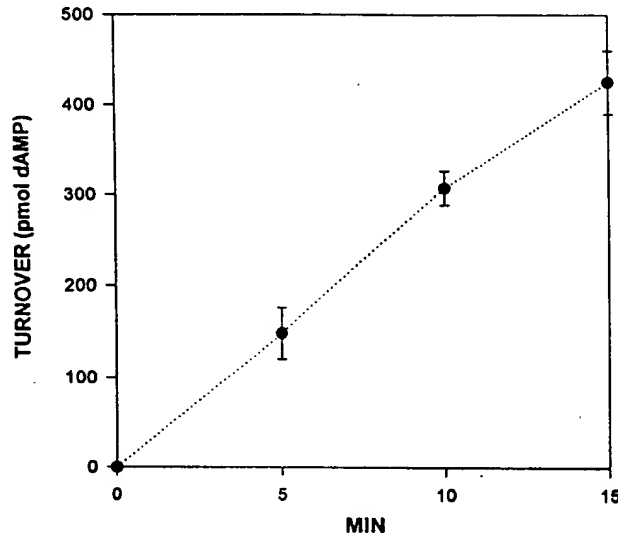
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24

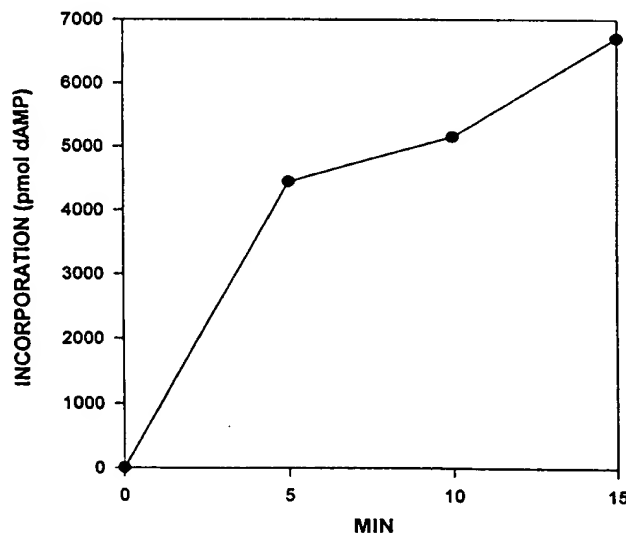
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PROOFREADING: TFI/VENT



- 4/15/95
cc
- Background was 118 pmd, so the best signal to noise occurs at 15 min (3.6x background). However, by 15 min, incorporation is slowing down as gaps are filled in. At 15 min some turnover is occurring at nicks - not a good model of a PCR reaction.
 - Trade off between good model and linearity of the time points is good.
 - A 10% drop in activity would be detected by this assay, using 5 replicates.

POLYMERIZATION: TFI/VENT



- Incorporation falls off after 5 min, because gaps are filled by high TFI polymerization activity, after that, turnover occurs at nicks.
- % turnover increases because DNA synthesis is slowing down while turnover keeps going at the same rate.

T Pag 1

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Invented by

Date

Dorinda Polanco

6/26/95

Recorded by

Paula Pomb

6/25/95

ag N —	Turnover (pmoles) dAMP	% Turnover
$\frac{\text{cpm} - \text{background cpm}}{\text{specific activity}} \left(\frac{30}{10} \right) \left(\frac{100}{2} \right)$		$\left(\frac{\text{pmoles dAMP}}{\text{pmoles incorporation + dAT}} \right) \times 100$
CPM1 = dAMP: dATP = dAMP 1776.00 86470.00	ex. $\left(\frac{1776 - 807}{465} \right) \left(\frac{30}{10} \right) \left(\frac{100}{2} \right) = 208$	ex. $\frac{208}{208 + 936} \times 100 = 18.2$
26' 2994.00 - 86141.00	470	21.2
30' 4209.00 - 91512.00	732	25.1
40' 4983.00 - 85588.00	898	24.7
50' 6822.00 - 94359.00	1290	32.1
60' 7013.00 - 85869.00	1330	33.3
70' 1216.00 - 79679.00	88.0	13.9
80' 2179.00 - 83426.00	295	24.0
90' 2954.00 - 81631.00	462	25.0
100' 3716.00 - 83944.00	623	26.6
110' 4469.00 - 85258.00	788	29.8
120' 5283.00 - 87259.00	963	30.3
130' 1223.00 - 85430.00	89.5	18.7
140' 1807.00 - 90067.00	215	25.2
150' 2316.00 - 88894.00	325	27.5
160' 2953.00 - 84914.00	462	30.6
170' 3572.00 - 90268.00	595	30.2
180' 3815.00 - 92711.00	697	33.0
190' 742.00 - 81173.00	\bar{x} dAMP = 807 cpm is background = 174 pmoles	
200' 749.00 - 87079.00		
210' 655.00 - 87371.00		
220' 890.00 - 86383.00		
230' 785.00 - 86929.00		
CPM1	$\text{specific activity} = \frac{929,462 \text{ cpm} \times 100 \mu\text{l}}{10 \mu\text{l}}$ $\frac{(5000 \text{ pmoles})(4)}{(5000 \text{ pmoles})(4)} = 464.7 \text{ cpm}$ pmoles nt DNA	
240' 978.00 - 90674.00		

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	Record d by _____		

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30

From Page No. _____	Results:	Relative mobility, $RM = \frac{\text{distance from origin to center}}{\text{distance from origin to 50}}$			
4/20/95 CSC					
SAM	CPM1	distance (cm) from origin to center of rectangle	RM		distance from origin to 50
			$.45/9 = 0.072$		
no enzyme, front at 9cm					
1	21456.00	0.65	0.17		
2	436.00	0.85 1.5	0.21		
3	217.00	1.65 1.9	0.26		
4	128.00	2.3	0.30		
5	105.00	2.7	0.41		
6	232.00	3.65	0.54		
7	73.00	4.9	0.66		
8	68.00	5.9	0.77		
9	78.00	6.9	0.91		
10	88.00	8.2			
60' vent, front at 9cm		same data as above			
11	18209.00				
12	572.00				
13	153.00				
14	87.00				
15	175.00				
16	1759.00				
17	119.00				
18	78.00				
19	151.00				
20	160.00				
21	17132.00	same data as above			
22	42.00				
23	46.00				
24	99.00				
25	314.00				
26	2236.00				
27	60.00				
28	72.00				
29	98.00				
30	174.00				
31	21668.00		0.077		
32	250.00	2.25	0.17		
33	175.00	2.75	0.21		
34	112.00	3.25	0.25		
35	115.00	3.75	0.29		
36	201.00	5	0.38		
37	63.00	6.5	0.5		
38	71.00	8	0.62		
39	101.00	10	0.77		
40	100.00	12	0.92		
41	17710.00	same			
42	326.00				
43	133.00				
44	125.00				
45	104.00				
46	1786.00				
60' vent, front at 13cm					
47	59.00				
48	56.00				
49	155.00				
50	121.00				
no enzyme, front at 18cm					
51	16715.00	same			
52	400.00				
53	225.00				
54	82.00				
55	91.00				
56	2316.00				
57	76.00				
58	60.00				
59	100.00				
60	194.00				
61	21188.00	1.25			0.1
62	499.00	2.75			0.1
63	195.00	3.25			0.1
64	86.00	3.75			0.1
65	83.00	4.25			0.1
66	99.00	4.75			0.1
67	81.00	5.25			0.1
68	75.00	5.75			0.1
69	139.00	7.25			0.1
70	73.00	9.25			0.1
71	66.00	11			0.1
72	53.00	13			0.1
73	130.00	16			0.1
74	19038.00	same			
75	333.00				
76	136.00				
77	79.00				
78	59.00				
79	90.00				
80	59.00				
81	73.00				
82	1811.00				
83	70.00				
84	50.00				
85	55.00				
86	262.00				
87	18132.00	same			
88	449.00				
89	249.00				
90	72.00				
91	56.00				
92	76.00				
93	70.00				
94	66.00				
95	2231.00				
96	99.00				
97	45.00				
98	68.00				
99	234.00				

T Pag

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Invent d by

Dat

6/26/95

6/20/95

R c rd d by

David Rumb

Deena Polansky

Primer degradation by TFI/Vent - Epicenter
Time course & 5 replicates on 12% gel

Book No. _____

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purpose: To measure 3'→5' exonuclease activity of TFI/Vent mix using the primer degradation assay. The data will be part of the stability study on the mix. The following changes to the assay will be made in hopes of obtaining more accurate data on the rate of primer degradation:

- 1) 12% gel will be used instead of an 8% gel - may give better peak resolution. Last time, peak shoulders and double peaks were a problem during quantitation.
- 2) 44mer^{Fidel} will be used instead of 33mer - correct - the 33mer could form primer dimers &/or a hairpin that may have altered the degradation rate.
- 3) By doing 5 replicates, we can assess the accuracy of this assay as compared to the turnover assay.

Primer degradation by Vent alone will also be measured.

Background: Note - although we tested TFI/Vent mixes on p. 9 NB11, only the TFI purchased from Epicenter is TFI. LTI's TFI is really Tth. That is why we are not doing any stability tests on LTI's enzyme, until a new TFI alone is obtained.
- the amount of enzyme and time course of this experiment are known to be in linear range of assay from the earlier expt on p. 9 NB11.

materials: $\gamma^{32}P$ -end labeled 44-mer Fidel

fresh mix A

12% denaturing sequencing gel

stop 50% EtOH

TFI (Vent) mix made w/ TFI purchased from Epicenter
Vent diluted w/ TFI Epicenter SB, Vent 10x & 17

recipe for 12% gel, 100mL:

48g urea
30mL, 40% acrylamide:bis mix
10mL, 10x TBE
dissolve by stirring & low heat
+ 600uL 10% AP (100mg/L)
qs to 100mL w/ H₂O - squirt bott.
30uL TEMED
100mL

To Page No. _____

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6/26/95

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Carolyn Combs

Date

6/21/95

From Page No. _____

mix A: 90ul per rxn, enough for ¹⁵ rxns

$$6ul \times 9 = 54ul \text{ H}_2\text{O} \quad 990ul$$

$$20ul \times 9 = 180ul \quad 5x \text{ Cheng buffer } 300ul$$

$$4ul \times 9 = 36ul \quad 32P\text{-}44mer \text{ Fidel, } 5uM \text{ stock } CF=2000$$

$$90 \times 9 = 810ul \quad 1350$$

procedure:1) end label the 44-mer Fidel primer - 70.3ul H₂O

24ul 5x Kinase buffer

6ul 44mer Fidel, 100uM stock

13.7ul ³²PγATP, 10uCi/ul
ref. date = 6.

6ul PNK 1u/ul

120ul

37°C, 30min

55°C, 5min

store at -20°C overnight

2) deliver 5ul stop soln to 9600 tubes ~~1-28~~ / label rxn tubes 1-
make mix A

rxn #	1	2	3	4	5	6	7	8	9	10	11	12
H ₂ O	8	8	8	8	8	8	8	8	8	8	8	8
mix A	90	90	90	90	90	90	90	90	90	90	90	90

preheat to 65°C in 9600, start by adding e

Cp 50*
no enzyme
8ul

2ul TFI / Vent - Epicenter 2ul Vent.

At each time pt remove 10ul rxn to the 5ul stop soln
- heat 90°Cdo { no enzyme 0', 10' } replicas: 4', 6' at 70°C
time course 2', 4', 6', 8', 10', 20' w/ TFI / Vent

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D. Polans

6/26/95

R corded by

Dawson Pomb

4/21/95.

age N _____

picenter SB for no enz control: final 100mM NaCl, add solid to LTT SB
and to dilute vent with.

final 50mM Tris

now at 20mM

5 mL Taq SB = LTT SB ✓

91.4 μL 1M Tris 7.5 - pre-made by LTT ✓

62.5 μL 1M Tris-HCl ✓ → 157.649/mole

153.5 μL glycerol ✓

0.031 g NaCl (58.449/m) ✓

$$\frac{1 \text{ mole}}{1} \times 10 \times 10^{-3} \text{ L} = 0.01 \text{ mole}$$

$$\frac{\text{g}}{157.649/\text{m}} = 0.01 \text{ mole}$$

$$= \frac{1.5764 \text{ g Tris-HCl}}{+ 10 \text{ mL dH}_2\text{O}}$$

Vent dilution in Epicenter storage buffer = 22.2x dilution to 0.094 μL
2 μL Vent stock (2 μL/μL) w/ p2 pipetman → 1st spin down + vortex Vent stock
+ 42.4 μL Epicenter storage w/ p200 pipetman
44.4 μL vortex to mix

samples were heated to 90°C, 5' in 9600 prior to loading.

sample #3

1 = no enz 0 min

2 = no enz 10 min

3 = TFI/Vent 2' min - not preheated = may be off

4 = " 4' 21 4 > 2

5 = " 6' 22 6 > 2

6 = " 8' 23 4 > 3

7 = " 10' 24 6 > 3

8 = " 20' 25 7 > 4

9 (1) TFI/Vent 4' 26 4 > 1

10 " 6' 27 4 > 5

11 (2) 4' 28 6 > 5

12 6' 29 6 > 5

13 4' 30 4 > 3

14 6' 31 6 > 3

15 4' 32 4 > 4

16 6' 33 6 > 4

17 4' 34 4 > 5

18 6' 35 6 > 5

19 Vent 4' 36 4 > 1

20 6' 37 6 > 1

2nd load 1, 3-28, 12 2nd run longer
1st load 1, 2, 4-28, 2 1st
1, 29-28, 2 no times course

To Page No. _____

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Solamp

Date

6/26/95

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Paula Corns

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From Page N. _____

sample #

1st loading: 1, 9-28, 2 (no time course) ~ 3:15^{pm}, rate $\frac{0.7 \text{ cm}}{8 \text{ min}} \approx 0.1 \frac{\text{cm}}{\text{min}}$

2nd loading 2, 3-28 w/ time course ~ 5:15^{pm}, run until β reaches bottom. At this time ~ 10 bases will have run. This will be our whole gel loading to see the whole gel most of the products

Gel was run at 1700V constants for a total of 5.5 hrs.

T Pag N.

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6/26/95

Invented by

Recorded by

D. J. O'Keefe

Date

6/21/95

The ad Tag with Cheng buffer
for gap DH P

Project No. _____
Book No. _____

ig N	1	2	3	4
5 x Cheng buffer (w/ dNTPs) (see P20, 10)	10	10	10	10
10 mM dNTPs	1	1	1	1
Human spleen genomic DNA 80 ng/μl	1.25	—	—	→
2112 12 μM	1	—	1	—
2113 10 μM	1	—	1	—
5' u/μl Tag	0.5	0.5		
The 5' u/μl H ₂ O	35.25 50 μl	33.25	0.5 35.25	0.5 33.25

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OB Olamp

Date
6/26/95

Invented by
Recorded by

Date
6-23-95

To Page No. _____

ig N .

SigmaPlot regression lines:

1) for Proofreading: TFI/Vent the slope = $28.676 \frac{\text{pmole}}{\text{min}}$, $r^2 = 0.978$

2) for Polymerization: TFI/Vent the slope = $417 \frac{\text{pmole}}{\text{min}}$, $r^2 = 0.873$

Units of TFI/Vent proofreading: $28.68 \frac{\text{pmole}}{\text{min}} \times \frac{30 \text{ min}}{10,000 \text{ pmole}/\mu\text{l}} = 0.043 \mu\text{l}$

Units of TFI/Vent polymerization: $417 \frac{\text{pmole}}{\text{min}} \times \frac{30 \text{ min}}{10,000} / 2 = 0.63 \mu\text{l}$

expect: $0.045 \mu\text{l}$

conclusions

1) The turnover assay can detect a 10% loss of 3' exo activity in TFI/Vent mixes. By repeating the assay more frequently and/or with more replicates the error may decrease so that a 5% loss of activity could be detected.

2) Early time points, before 10 min, reflect turnover during DNA synthesis - the best model of PCR. Later time points reflect turnover during DNA synthesis plus turnover at DNA nicks - not such a good model of PCR. However, the later time points give better data because the signal to noise ratio is higher (3.6x versus ~1.3x early on). Both all 3 time points should probably be done during the stability study.

3) Turnover by TFI/Vent mix is about 3x higher than by Vent alone. This result was observed in an earlier experiment too. TFI may create more mismatches for Vent to turnover than when no TFI is present.

↑
not true. error in TFI/Vent is only ~ 2x higher than for Vent alone (see mismatch on P 40, 10). In this experiment (P 17, 11) the Vent alone of 2 μl of $0.1 \mu\text{l}/\mu\text{l}$ is the one we should compare to the TFI/Vent mix.

To Page No. _____

and Understood by me,

Ernest C. Polansky

Date

6/26/95

Invented by

Recorded by
Cawlyn Combs

Date

6/24/95

PLATES

Both the dATP + dADP peak and dAMP peak become more spread out in the PEI plates as the solvent front runs further. The distance between the two peaks becomes greater as the solvent front runs further: ~0.5cm vs 2cm

Conclusion: For best resolution of dAMP from dATP + dADP on PEI plates, run the LiCl solvent front to the top of the plate, ~16 cm from origin.

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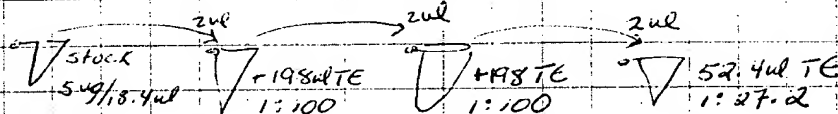
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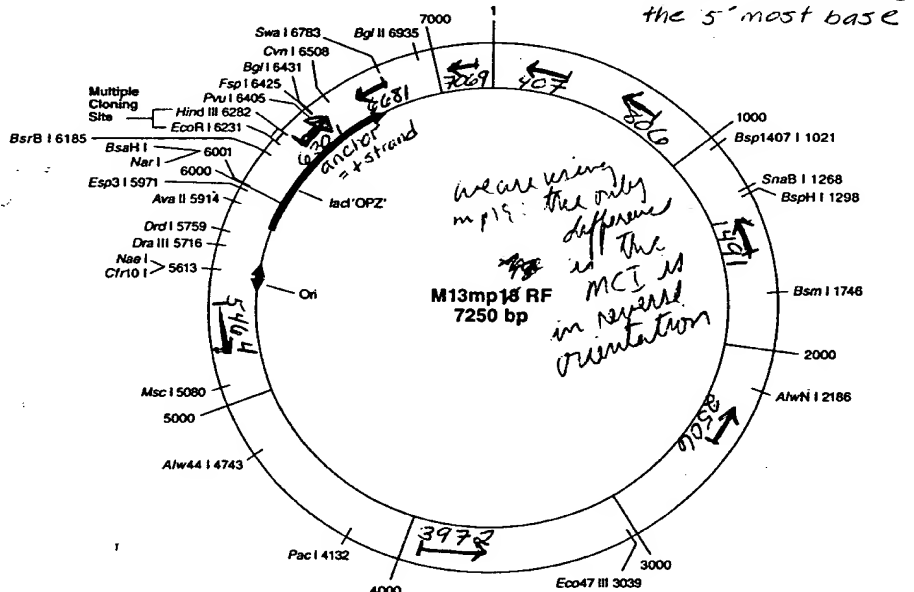
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Dilution of M13 RF to 1 pg/ul in TE: stock lot CN4132, 5 ug/18.4 ul
do serial dilutions to $1/2.72 \times 10^5$



6/20/95

M13mp19 prime

→ 20mers

→ 9G+C

→ 11A-T

→ $T_m = \sim 58^\circ\text{C}$

primer name

PCR product length (bases)

sequence
GTCGTG

M13-6301 anchor (= + strand)

5' GTTTTACAAC

M13-6681 (= - strand)

380

5' TTCC TGTAGCCAGCTTTC

M13-7069 "

768

5' ATG CCTGAGTAATGTGTAGG

M13-407 "

1356

5' GAAGCAAAGCGGATTGCA

M13-806 "

1755

5' TTA TACCAATCAGGACGT

M13-1491 "

2440

5' AGCTTGATACCGATAGTTGC

M13-2506 "

3455

5' CGACAGAAATCAAGTTTGCC

M13-3972 "

4921

5' AATCGCAAGACAAAGAACG

M13-5464 "

6413

5' GTATAACGTGCTTTCTCC

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[Signature]

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separation of primer stocks and dilutions:

Primers were made by Gibco BRL Custom Primers order# 510790A

Each primer was ~~that~~ resuspended in sterile dH₂O at a CF=100µM
- spun down, H₂O added, 2min RT, vortex, invert

primer	nmols/tube	volume of dH ₂ O added to make CF=100µM
M13-6301 anchor	54.7	547 µL
M13-6681	44.09	440.9
M13-7069	39.14 57.29	391.4 572.9
M13-407	39.14	391.4
M13-806	51.92	519.2
M13-1491	69.49	694.9
M13-2506	66.34	663.4
M13-3972	34.68	346.8
M13-5464	42.45	424.5

Each 20 µM aliquots of each primer were made from the 100 µM stocks:

⇒ 1:5 dilution, 40 µL of 100 µM primer stock

+ 160 µL dH₂O-sterile

200 µL for each primer except M13-6301 anchor

1:5 dilution of M13-6301 anchor, 200 µL of 100 µM stock

+ 800 µL dH₂O-sterile

1 mL

inuation of PCR expt from p 40

10 µL of 1 Kb ladder and 10-18 µL of PCR products were run on a 1% TAE agarose gel at 190 V (~180 mA, 40 W)

1% gel recipe: 220 mL 1x TAE

buffer: 2 L 1x TAE

2170 µL CTBr at each end

2.2 g agarose

wt = 435.6 g, boil, reweigh & add H₂O back to orig. wt

stir & cool

add 15 µL, 10 mg/mL CTBr & pour into rig. wt quarters

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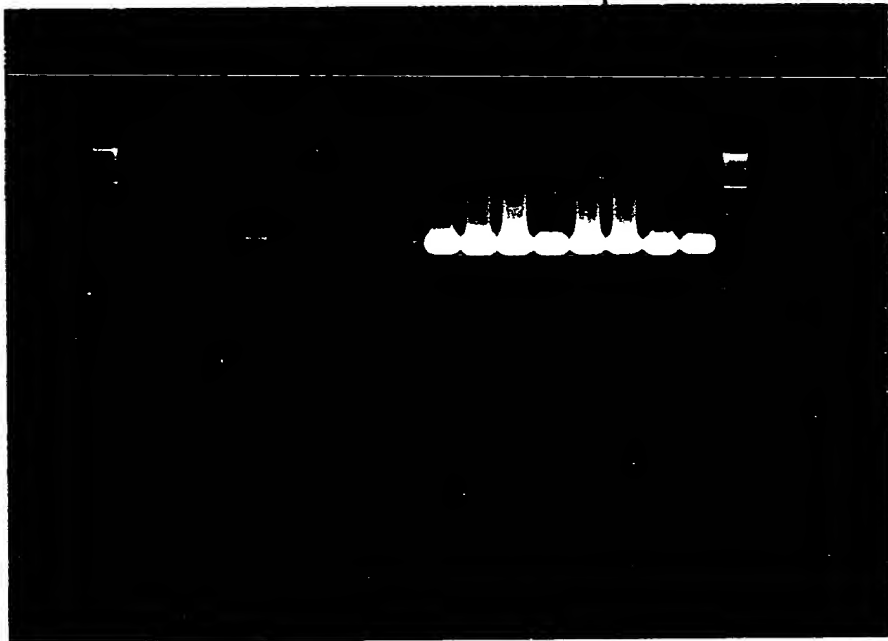
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Carolyn Lamb

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taped into
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> Since 10ul of the rTa
PCR rxns contained suc.
low level of product,
tried loading 15ul in or
to be able to visualize
products better

> No specific products
seen in the Tne. lat

taped into book
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> note false product
1491 primer
- only 1 band of 2
was expected
- instead there are 2
~ 0.5-1 Kb and 2-3

> Note false product
m13 2506 primer -
should be ~3.5 Kb
> also a short, false
product w/ m13-5464

T Pag

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Date

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Date

M. Davis

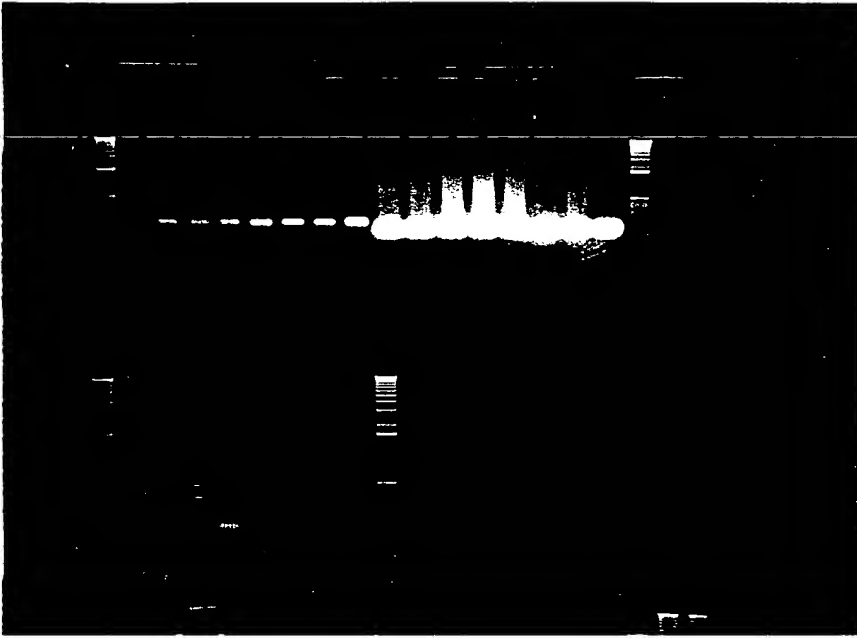
6/30/95

Recorded by

David M. Pomb

6/26/95

ig N _____



> 6' extension time did not
give more product. - worse
yield than with 2' extension

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cc book 6/30/95

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JS

Date

6/30/95

Invented by

Recorded by

Caulyn Combs

Date

6/26/95

M13 PCR system: optimizing annealing temperature

ag N _____

[A]

(2 Runs)

LTI 10X ~~25~~ PCR buffer

120 ✓

50 mM MgCl₂

36 ✓

4 dNTPs 10 mM each

24 ✓

M13 RF 1 pg/μl (lot # CN4132)

12 ✓

M13 6301 "Anchor" 20 μM

24 ✓

rTag 5 μ/μl

12 ✓

H₂O

947 ✓

1.176 mL

95

51, 49, 47°C annealing temp w/ 1st 3 oligos program 19, 94°C
18, 94, 15"
1 2 3 4 5 6 7 8 9 - 51, 3"
70, 2"

[A] 97 μl →

36671 2 2 2 ✓
37065 2 2 2
3407 2 2 2

V_f = 100 μl

14 9600 51°C annealing temp 1-3
15 49°C 4-6
16 47°C 7-9

1% gel: 2.64g agarose + 220mL
220mL

100k
100k
ladder

100k
100k
ladder

3.36g agarose + 280mL TAE wt = 480.3g

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Read & Understood by me,

Bob Long

Date

6/30/95

Invented by

Record d by

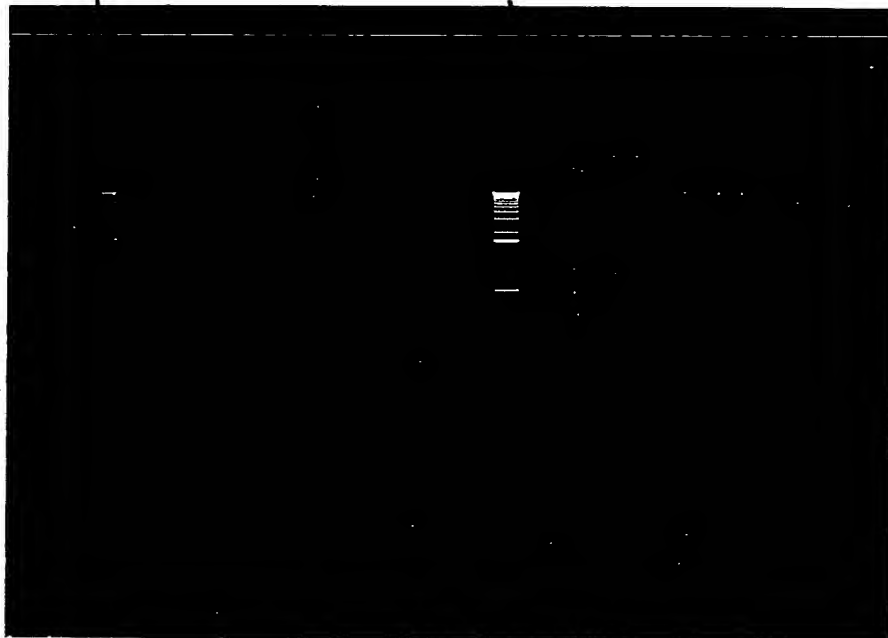
Emily Pomeroy

Date

6/24/95

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Result:



6/26/95 cc

primer combo	expected product length (bp)	observed product length (bp)
anchor + 6681	380	✓ looks like 380 relative
anchor + 7069	768	✓ 768 "
anchor + 8407	1356	✓ 1356

> specific products of the right length were made, but the yield was still v. low. Cole thought lowering the annealing temp might result in a higher yield. It did not. The lanes on the right side of the gel just look a bit darker because the light box is brighter on that side (note how the 10kb ladder looks more intense on right side even though 10ul was load on left & right sides. Also note

> Next we'll try to increase the yield by using denaturation time (from 15" to 30" - Veri's suggestion), [Taq], Δ cycle & [primer] - in c the anchor primer has a hard time annealing due to 2° struc. If it does, then lowering annealing temp would exacerbate the problem.

T Pag No

With ssed & Und rst d by m ,

Date

Inv nt d by

Dat

N. O. O. O.

6/30/95

R corded by

P. O. O. O.

6/26/95

Δ denaturation temp, Δ [Tag]
 Δ cycle number, Δ (primer)

Project No. _____
 Book No. _____

g No. _____

10x PCR buffer
 50 mM MgCl₂
 4 dNTPs 10 mM
 M13 RF (lot FAS701) 1 pg/μl
 opened 6-26-95
 H₂O

[A]
 100 ✓
 30 ✓
 20 ✓
 10 ✓
 740 ✓
 900 μl

different lot than on p. 40
 dilution of stock

$\begin{matrix} \text{stock} & \xrightarrow{2\mu\text{l}} & & \xrightarrow{2\mu\text{l}} & & \xrightarrow{10\mu\text{l}} \\ 370\mu\text{g} & \text{H}_2\text{O} & & \text{H}_2\text{O} & & \text{H}_2\text{O} \\ \text{ml} & & & & & \end{matrix}$
 $\frac{1}{100} \quad \frac{1}{100} \quad \frac{1}{37}$
 = 3.7×10^5 fold dilution

#	1	2	3	4	5	6	7	P
	90 μl							
	5' 1' 4'				5' 1' 4'			
	4P 4P				4P 4P			

1:1 mix of anchor + 407
 30 μl 20 μM stock anchor
 30 μl 20 μM 407
 60 μl, 10 μM each

(400 or 800 nM oligo each)

5' μl 1 1 2 2 1 1 2 2 ✓

74°C, 1' initial denaturation

15" denaturation
 94°C

↓
 53°C, 30 sec

↓
 70°C 2 min
 Lab 15, 9600

30 sec denaturation
 94°C

11

11

Lab 16, 9600

30 and 40 cycles 3:15 PM
 5.20 μl rxn 1-8
 + 2.3 μl 10x LD
 Freeze ON
 left in PCR machine, at 4°C ON

To Page No. _____

d & Understood by me, stamp	Date 6/30/95	Invented by 	Date 6-26-95
		Recorded by Carolyn Cont	

Project No. _____

Book No. _____

TITLE _____

Results P. 49

From Page No. _____

Denaturation time

15 sec

30 sec

units Tag

5

10

5

10

primer (nM)

400

700

400

700

400

700

400

700

cycles

30 38

30 38

30 38

30 38

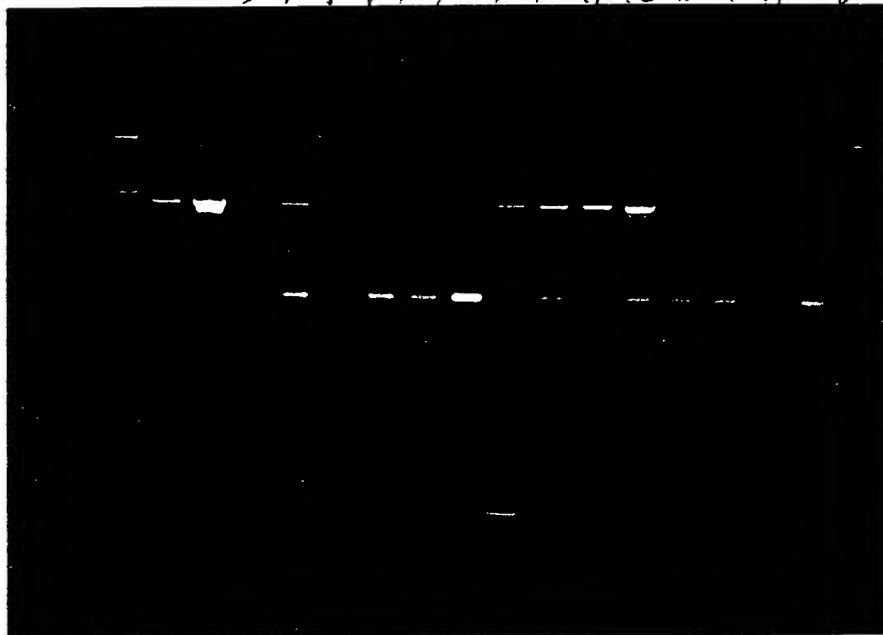
30 38

30 38

30 38

30 38

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16



E 1356

← primer dimer

- . tube 1 compared to tube 3 on 49 shows new M13RF only gives little or no improvement to yield at 30 cycles
1. lower [primer] (400 nM) is best (tubes 1, 2 vs 3, 4) (when denaturation is for 30 sec, 400 vs 700 nM primer about equal)
 2. Lower Tag is best: only primer dimer made for 10 (# 5-8 and 13-16)
 3. 38 cycles made more product than 35 cycles (tube 2 vs 1)
 4. 30 sec denaturation gave less product than 15 sec.

T Pag N.

With ss d & Und rst d by me,

Dat

6/30/95

Invent d by

R cord d by

Random Pomb

Dat

6-26-95

R. O. L. O.

Page No. _____

Therefore:

keep 15 sec denaturation

Try even less primer (eg 100 200 300 400 nm)

Try even less tag (2, 3, 4, 5 min)

Try different cycle number (30 - 40)

Try M13 6301 (anchor) alone and with other primers
with no target to look for primer dimerTry more M13 RF target
eg. 0.1 pmol - 10 nmolTry R1 (or Bam, Hind III etc) "check buffer salt
cut

PCR with no purification

Test all primers with best conditions

To Page No. _____

Read & Understood by me,

6/30/04

Date

6/30/04

Invented by

Recorded by

C. and J. N. only

Date

6-26-04

Exhibit L-117

Appl. No. 09/558,421

Project No. _____

Book No. _____

TITLE 4°C storage (see P121, 9) -20°C and -70°C ,
for freeze & thaw

52

From Page No. _____

	Rxn #	array	ul	
# 10 (P121, 9): no det	1-3	2	✓	This is 5 month point for 4°C studies (same as P121, 9; 154, 9; 174, 9; 37, 10 = 0, 1, 2, 4 m)
# 11 " 1.1X	4-6	3.64	✓	
amp Temp # 11: 1.1X	7-9	3.64	✓	(154, 9 is 0 time point, P 38, 10 is 1 month)
Tag # 125 did (same as P121, 9)	10-14	2	✓	
1.1X May 8, 1995	15-17	2	✓	called "new" on P 34, 10
1.1X field test	18-20	2	✓	called "old" P 34
1-27-95 -20°C	21-23	2	✓	Joel took aliquot from samples on 1-27-95 gave & freeze Thaw stored at -20°C , 5 months at with unknown effect from freeze/thaw
-20°C 5/24/95	24-26	2	✓	from 1.1X May 8, 1995 (compare to Rxn # 15-17 above) w/ month at -20°C with no extra freeze th
-70°C 5/24/95	27-29	2	✓	from 1.1X May 8, 1995 - its w/ month at -
* 10 freeze Thaw	30-32	2	✓	* used 1.1X May 8, 95 (above) in "new"
20 freeze Thaw	33-35	2	✓	drying EtOH $\rightarrow 30^{\circ}\text{C}$ bath 10
30 freeze Thaw	36-38	2	✓	start with 60 μl and take out at 10, 20, 30 freeze Thaw
Fr 13-17 5200 Tnl				
P 25, 10				
1/700	39	2	✓	
1/700	40	2	✓	
1/700	41	2	✓	
Tnl 5-7-95 ($\sim 70.7^{\circ}\text{C}$ on P 25)				repeat of P 25 for Tnl did 5200 fractions look activity at 4°C
1/8000	42	2	✓	
	43	2	✓	
	44	2	✓	
48 μl Tox unit assay mix (P120, 9) in each				
10' 74 $^{\circ}\text{C}$				
kill' with 10 μl 0.5M EDTA, spot on 6-FC				
				20 μl

To Page !

Witnessed & Understood by me,

J. D. Roberts

Date

6/30/95

Invented by

Recorded by

Date

6-28-95

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Appl. NO. 071550,421

B ok No.

Percent
of zero time
in P. 122/9

je N		u	u/a relative to 10g (#10-14)		
1	1597.00				
2	1752.00				
3	1760.00				
4	7709.00				
5	6150.00	6760 ave	.030	94%	
6	6422.00				
7	4510.00				
8	5085.00		.025	72%	
9	5662.00				
10	8620.00				
11	9351.00	8931 CPM ave	.04 (by definition)		
12	8531.00				
13	8321.00				
14	9832.00				
15	5618.00	5132 ave	.025	10.9%	
16	5895.00				
17	5384.00				
18	5128.00				
19	5036.00	5215 ave	.023 u/a	10.9%	
20	5481.00				
21	3989.00				
22	4058.00	3673 ave	.023 u/a	10.9%	
23	2971.00				
24	5931.00				
25	5591.00	5921	.023 u/a	10.9%	
26	6242.00				
27	5891.00				
28	5381.00	5712	.023 u/a	10.9%	
29	5865.00				
30	5644.00				
31	5407.00	5440	.023 u/a	10.9%	
32	5271.00				
33	5362.00				
34	5494.00	5405	.023 u/a	10.9%	
35	5361.00				
36	5556.00				
37	6159.00	5638	.023 u/a	10.9%	
38	5200.00				
39	138.00				
40	287.00				
41	137.00				
42	1014.00				
43	960.00				
44	1092.00				
45	395.00				
46	110131.00				
47	110429.00				

its been at -20°C for 1 month - 20°C from 5-8-95 (1.1X #15-17 above)
 3673 ave it came from tube #11 above (approx #4-6) conclude -20°C for activity
 5921 → 1 month -20°C from 5-8-95 (1.1X #15-17 above)
 Sample from 1.1X of 5-8-95 (activity 1.1X above) ⇒ no activity but for 1 month at -20°C
 5440 started with 1.1X (started only at 40°C) concluded no loss of units for 30 freeze Thaws
 5405 (of 5-8-95) (1.1X above)
 5638 The died at 40°C off 5200 col of 5-18-95 got 7.7 u/a on RST
 ave - RST = 62.7 CPM = 27.3 pma [32.8 u/a] agrees with 36 u/a
 L. g. Flynn got out 8. g. got P. 13. May 70. 7.7 u/a
 BKGD of P. 22 may be wrong The 1.1X stable until more data is available
 2x mix
 2x mix
 68.9 CPM/pma

ed & Understood by me, 10/10/95	Date 6/30/95	Invented by [Signature]	Date-30-95
		Recorded by [Signature]	(6-28-95)

3 batch:

①
17 sample
original buff. formulation -

created
40C RT -200C 27/1

* RT & NaN₃ *
1.1x
+ ~~original~~
*

* 8x
thaw
*

②
Date
field test

created
RT 40C *

May 8, 95 5

③
("new" on P34, 10)
500ul batch
lot

created
40C
24/5/95
add 1g each 1-2ul
-20C -70C RT 40C
* * *

thaw
make aliquots
20ul each
as -20C
only 1 or 2 freeze thaw

The unit array *

notes from
Joe Jolas

R Jolas
6-27-95

Turnover by TFI/Vent - 7 replicates of _____ Book No. _____
 Epicenters TFI/Vent and LTI's TFI/Vent in Epicenters SB - zero time of for stability
 ag N _____

pose: To establish the 3' exo activity of TFI/Vent by Turnover on
 gapped DNA at time zero of stability study. 7 replicates
 to reduce error

Background: The zero time point turnover assay has already been
 for Vent alone and LTI's TFI/Vent in LTI SB
 with 5 replicates NB11 page 17 and 21

pro to deliver 10ul stop & 10ul rxn-wipe tips

materials: mixA, enough for 40 rxns, each using 98ul of mixA ✓
 for 23 = 1467ul H₂O ✓
~~40 x 63.786 = 2551.44ul H₂O~~ → 4605x Cheng ✓
~~40 x 20 = 800ul 5x Cheng~~ → 310ul act DNA
~~40 x 13.5 = 540ul activated DNA~~ → 11.5^u dATGC-TP
~~40 x 0.5 = 20ul dATGC-TP, 10mM each~~ → 4.92ul ³²P dATP
~~40 x 0.214 = 8.56ul ³²P dATP~~ → 2253.422 ^{6/30} Ame
 3920ul

5	1-7	8-14	15-21	22-28	29-35
tube	1-21	22-42	43-63	64-84	85-91
	1-7	8-14	15-21	22-28	29-35
	1-21	22-42	43-63	64-84	85-91
x A	98ul	prewarm	to 68°C		43-49

Tvent (LTI SB)
 2ul

1 Vent (epi SB) 2ul

1 Vent (epicenter TFI)
 2ul

ent diluted (5-14.55)

2 enz

2ul LTI SB = Tag ^{5/5}
 To Page No. _____

Used & Understood by me,

Shulans

Date

6/30/95

Invent d by

R. J. ...
 Recorded by
Carolyn Combs

Date

6-29-95

From Page No. _____

At 5, 10, 15 min remove 20ul rxn to 20ul stop soln (P14)
 Spot 2ul on PEI plates
 Spot 10ul on GFC filters (2 per rxn) + 2ul mix A 3x

tube #s

1 2 3
 4 5 6
 7 8 9
 10 11 12
 13 14 15
 16 17 18
 19 20 21

epi
 epicenters Tfl enzyme + Vent in LTI SB
 (=Tth?)

22 23 24
 25 26 27
 28 29 30
 31 32 33
 34 35 36
 37 38 39
 40 41 42

LTI's Tfl eng in ~~at~~ epicenters SB + Vent
 =Tth

5 10 15
~~43-49~~

43-49 = no enzyme

GFC'S 1-6 = 2 replicates of epicenter eng + Vent
 7-12 = 2 replicates of LTI's eng in epicenter SB
 13 = no eng
 16-18 = 2ul mix A

To Page 1

With ss d & Underst od by m ,

Date

Inv nted by

Dat

R c rded by

6/30/95

6-29-95

J. Olson

Paulson Pomb

From Page No. — see p. 51

purpose: The PCR worked well (gave a large amount of product when 38 cycles were done using 400nM primer

5 uL/100 uL Taq,
15 sec denaturate

Now we'll try to get the same good plateau yield by optimizing [primer], [enzyme], checking for anchor problem [target] & linearizing target - using just 30 cycles

program 74 mix

expt 1. Δ [primer] from 100, 200, 300, 400 nM w/ 5 uL Taq, 15" dena 30 cycles

make **A**:
120 uL 10x LTI PCR buffer
36 uL 50 mM MgCl₂
24 uL 10 mM 4 dNTP
12 uL 1 pg/uL m13RF → dilute w/ TC
888 uL H₂O
1009 uL
1080 uL

run	1	2	3	4
A	90	90	90	90
H ₂ O	9	8	7	6
primer mix				

A: 50 uL 10x PCR buffer
15 uL 50 mM MgCl₂
10 uL 10 mM 4 dNTP
5 uL 1 pg/uL m13RF
395 uL H₂O
5 uL rTaq, 5 uL
480 uL

2 uL of 370 ug stock + 198 uL TE - mix
2 uL + 198 uL TE - mix
10 uL + 360 uL TE ⇒ 1 pg/uL

make primer mix: 6 uL 20 uM anchor primer
6 uL 20 uM 407 primer
12 uL 10 uM each

T Page 1

Withn ssed & Und rst d by me,

00000000

Date

6/30/95

Invent d by

R corded by

Paul M. Lomb

Date

8-25-95

ig N	Tube	1	2	3	4
0		0	1	2	3
mer mix		4	3	2	1
9		96	96	96	96

Tag] from 1 to 7 units / 100ul rxn → 400nm primers

30ul	10x PCR buffer	dilute rTaq 5x
24ul	50mM MgCl ₂	6ul rTaq 5 ^u /ul
16ul	10mM 4 dNTPs	+ 24ul Tag dilution buffer
8ul	1 pg/ul m13RF	30ul
16ul	20uM anchor primer	
16ul	20uM 407 primer	
584ul	H ₂ O	
744ul		

bx	5	6	7	8	9	10	11
3	93						
20	4	5	4	3	2	1	0
rTaq 1 ^u /ul	1	2	3	4	5	6	7
	100ul						

target DNA, 800nm primer, 10u Tag:

C	35ul	10x PCR buffer
	10.5ul	50mM MgCl ₂
	7ul	10mM 4 dNTPs
	242.5ul	H ₂ O
	7ul	rTaq (5 ^u /ul)

bx	12	13	14
er	4ul anchor	4ul 407	4ul anchor
	4ul 407		4ul 407
0	94	4	0
	92	92	92

To Page No. _____

sed & Understood by me,

20/10/95

Date

6/30/95

Inv nted by

Recorded by

Date

6-22/95

From Page No. _____

Δ [Target], use 400nM primers and 5u Tag 100ul
for 7 rxns

[D]: 70ul 10x PCR buffer ✓
21ul 50mM MgCl₂ ✓
14ul 10mM 4 dNTPs ✓ * dilute m13 RF to 0.5 pg/ul
14ul 20uM anchovy ✓
14ul 20uM 407 ✓
462ul H₂O ✓
7ul rTag 5u/ul ✓
20ul 1 pg/ul m13 RF
20ul TC ✓

Tube #	15	16	17	18	19	20
m13 RF (0.5 pg/ul)	1	1.5	2	6	10	14 ✓
H ₂ O	13	12.5	12	8	4	0 ✓
[D]	86	→ ✓				

RI digestion of template:

3ul 1 pg/ul m13 RF ✓
5ul 10x PCR buffer ✓
3ul 50mM MgCl₂ ✓
13ul H₂O ✓
1ul CcoRI 10u/ul ✓
25ul

37°C, 30'

8.3ul 8.3ul 8.3ul
91.7ul E →

3ul ✓
5ul ✓
3ul ✓
13' ✓
1ul H₂O ✓

37°C 30'

same ✓

1 pm - 1.50 pm

21 22 23

24

25

26

To Page 1

With ss d & Und rst od by me,

Dat

6/30/95

Inv nt d by

R cord d by

Dat

6-29-95

DB Olamp

Rochester Pamb.

Fig. N. —

4.6 μ l	10x PCR buffer	$\times 7$	=	32.2
2 μ l	10mM dNTPs		=	14 μ l
1 μ l	M13 RF	100 μ g/ μ l	=	7
83.1 μ l	H ₂ O		=	581.7
1 μ l	Taq	5 μ g/ μ l		7
<u>91.7 μl</u>				<u>641.9</u>

400 nmoles
300 nmoles
200 nmoles
100 nmoles

1 2 3 4 5 6 7

units Tag

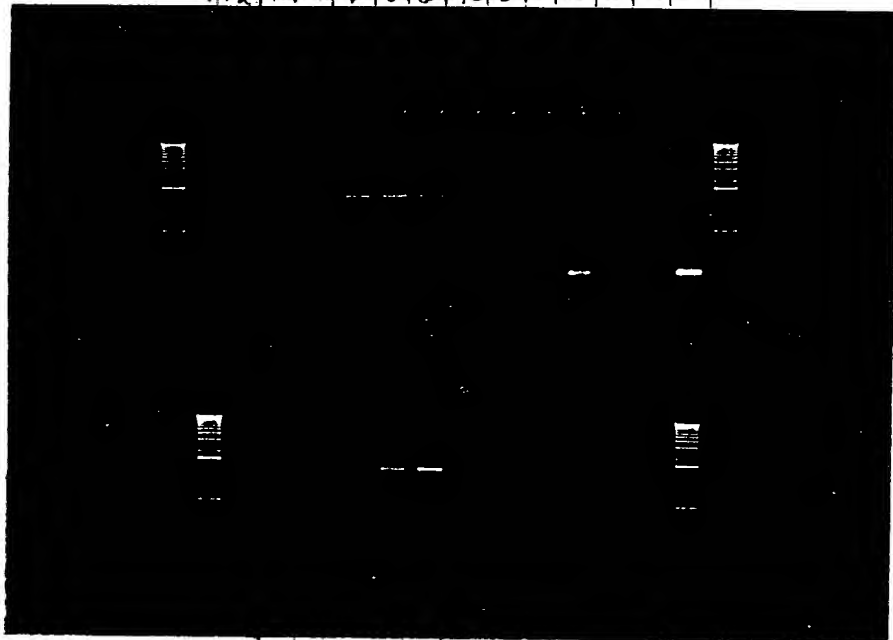
anchor
407
anchor + 407

no
ta

1 2 3 4 5 6 7 8 9 10 11 12 13 14

~~HT 2 3 4 5 6 7 8 9 10 11 12~~

anchor & 407 are primers



15 16 17 18 19 20 21 22 23 24 25 26

.5 .75 1 3 5 7 + C_{COPI} - C_{COPI}

pg target

To Page No.

ed & Understood by me,

Date

Invented by

DateRecorded by

folanges

$$6 \mid 30 \mid 90$$

Date
6-25-15

From Page No. _____

Results -

1. lag is inhibitory. Best results at 1 and 2 units
for 100 μ l PCR.
2. yields improve with increasing target
3. both primers ~~present~~ ~~is~~ ~~to~~ are required
to make primer dimers
3. its not happening substrate or one prime
~~self~~ annealing to another copy of
the same primer.

conclude the anchor primer should be OK
for most PCR's

Witnessed & Understood by me,

Date

6/30/95

Invented by

Recorded by

Date

6-2595

To Page 1

23mer. mp19 ssDNA

Project No. _____

Book No. _____

63

ge N (see P17, 9)

213 mp19 0.26 μ g/ μ l
 .79 nmol nt/ μ l
 .109 pmol circle/ μ l

200 μ l

226. (157 nmol nt total)

21.7 pmol circle
 0.594 nmol nt/ μ l total
 43.5 pmol primer total

23mer 5 ng/ μ l
 2.66 pmol primer/ μ l

66.1 μ l

266 μ l

23mer
 mp19
 circles = 2

70°C, 5 min

cool at room temp 40 min

(for 60 μ l 23. mp19)
 conc in 100 μ l Rxn

mp19 is 10 mM Tris pH 7.4
 5 mM NaCl
 0.1 mM EDTA

6 mM Tris pH 7.4
 3 mM NaCl
 .06 mM EDTA

note Cheng 1X (P20, 10) =

20 mM Tris pH 9
 7.5 mM KOAc
 2% DMSO
 1.05 mM MgOAc
 7% glycerol

So buffer in 23. mp19 will alter the reaction
 condition a little since 60 μ l 23. mp19 is needed
 per 100 μ l Rxn

in future need more concentrated DNA

To Page No. _____

Read & Understood by me,

Date

Invented by

Date

30/10/95

6/30/95

Recorded by

6-29-95

Results P.52

Percent
of zero time
on P122/9

ag	No.		u	u/a relative to 10g (#10-14)	100% zero time on P122/9	
	1	1597.00				
	2	1752.00				
	3	1760.00				
	4	7709.00	} 6760 ave	.030	94%	
	5	6150.00				
	6	6422.00				
	7	4510.00		.025	72%	
	8	5085.00				
	9	5662.00				
	10	8620.00	} 8931 CPM ave	↑ .04 (by definition) ↓		
	11	9351.00				
	12	8531.00				
	13	8321.00				
	14	9832.00				
	15	5618.00	} 5632 ave	$\frac{5632}{8931} = .04$.025	
	16	5895.00				
	17	5384.00				
	18	5128.00	} 5215 ave	$\frac{5215}{8931} = .04$.023 u/a	
	19	5036.00				
	20	5481.00				
	21	3989.00	} 3673 ave	its been at -20°C for 1 month + 6 Free Thaws it came from tube #11 above (Pipes #4-6) conclude -20°C losses activity 1 month -20°C from 5-8-95 1.1X (#15-17 above)	54% agrees from .05 u/a measured above (#4-6)	
	22	4058.00				
	23	2971.00				
	24	5931.00				
	25	5591.00	} 5921			
	26	6242.00				
	27	5891.00	} 5712	Sample from 1.1X of 5-8-95 (see tubes 15-17 above)	no activity lost for 1 month at -70°C	
	28	5381.00				
	29	5865.00				
	30	5644.00	} 5440	started with 1.1X (stored out at 40°C) of 5-8-95 (see #15-17 above)	97%	
	31	5407.00				
	32	5271.00	} 5405		conclude no loss of units for 50 Free Thaws	96%
	33	5362.00				
	34	5494.00	} 5638		100%	
	35	5361.00				
	36	5556.00				
	37	6159.00				
	38	5200.00				
	39	138.00				
	40	287.00				
	41	137.00				
	42	1014.00				
	43	960.00				
	44	1092.00				
	45	395.00				
	46	110131.00				
	47	110429.00				

ave - BKGD = 62.7 CPM = 27.3 pmol

BKGD 2x mix

2x mix

The died at 4°C off 500 u/a of 5-18-95
got 7.7 u/a on P125

agrees with 36 u/a
L. eg Flynn got and J. got P13. May 70. 7 u/a
of P125 may be wrong. will conclude L. & J's
The is stable until more data is available

54% agrees
from .05 u/a measured
above (#4-6)

101%

97%

96%

100%

32.8 u/a

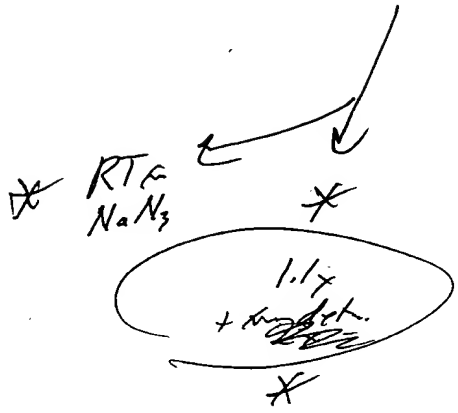
68.9 CPM/pmol

ave - BKGD = 627 CPM = 27.3 pmoL [32.8 u/μl] agrees with 36 u/a
L-ig Flynn got out & got P.13. May 70.7 u/a
BKGD of 2.2 may be wrong. The is stable until more data is available
2.2 mix
2.1 mix
[68.9 CPM/pmoL]

3 batch?

①
17 sample
orig. diff. formulation -

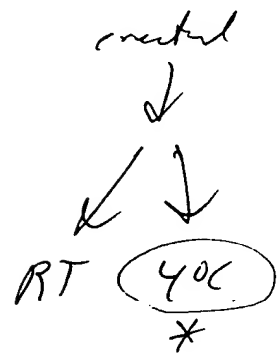
created
40C RT -200C 27/1



8x
thaw
*

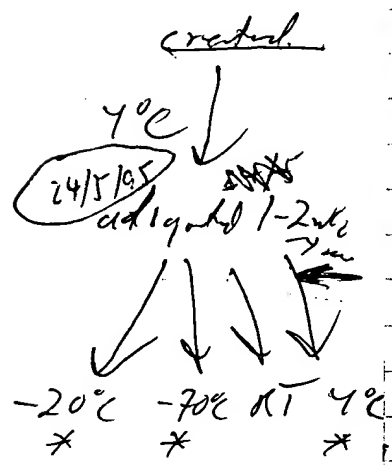
The unit array *

②
Date
field test



May 8, 95 5

③
("new" on P34, 10)
500ml batch
lot



thaw
make aliquots
20 µl each
at -20°C
(only 1 or 2 freeze thaw)

notes from
Joe Jones

R. Jones
6-27-95

ag N — specific activity = $\frac{(109,973 \text{ cpm}) \times 100 \mu\text{l rxn}}{2 \mu\text{l spot}} = 275 \frac{\text{cpm}}{\text{pmol (nt)}}$
 background = 188 cpm (5000 pmol) 4

Turnover (pmol)
 $\frac{(\text{cpm} - \text{background cpm})}{\text{specific activity}} \left(\frac{100}{2} \right) \left(\frac{20}{70} \right)$

CPM1

5' 774.00
 10' 1379.00
 15' 2170.00
 5' 1031.00
 10' 1588.00
 15' 2241.00
 5' 893.00
 10' 1731.00
 15' 1890.00
 5' 788.00
 10' 1365.00
 15' 1836.00
 5' 752.00
 10' 1055.00
 15' 1732.00
 5' 636.00
 10' 1140.00
 15' 1448.00
 5' 854.00
 10' 1458.00
 15' 2083.00
 829.00
 1512.00
 2124.00
 980.00
 1612.00
 2249.00
 1182.00
 2028.00
 2271.00
 1040.00
 1816.00
 2521.00
 944.00
 1729.00
 2032.00
 1087.00
 1641.00
 2701.00
 917.00
 2146.00
 2530.00

$\frac{774 - 188}{257} \times 100 = 228 \quad 213$

433
 721
 307
 509
 747
 256
 561
 619
 218
 428
 599
 205
 315
 561
 143
 346
 458
 242
 462
 689

$\bar{x}_{5'} = 229 \pm 45 \quad (20\%) \rightarrow$ Large error, next time
 $10' = 436 \pm 86 \quad (20\%)$ cut out dATP + dADP
 $15' = 627 \pm 101 \quad (16\%)$ spot in order to correct
 for spotting error

$\bar{x}_{5'} = 293 \pm 45 \quad (15\%)$
 $10' = 580 \pm 84 \quad (15\%)$
 $15' = 785 \pm 88 \quad (11\%)$

no change

195.00
 162.00
 169.00
 194.00

186.00
 189.00
 223.00

background for turnover
 $n = 7 \quad \bar{x} = 188.3 \pm 19.8 \quad (\sim 10\% \text{ error})$
 $= 6.8 \text{ pmoles}$

To Page No. _____

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Penelope C. mls

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6/30/95

Fr m Page No. ^{adjust V_f}
 back to 2661
 23. mp19 0.164 ^{1.95} $\mu\text{g}/\mu\text{l}$
 0.594 nmol nt/ μl
 P. 63
 5x Chevy (P21, 10)
 4 dMP₂ 10 mM each
 2 ³²P dATP 10 mCi/ μl (Amersham)
 H₂O

mix A
 264 μl 264 ✓
 88 μl 108 ✓
 2.2 μl 2.7 ✓
 1 μl 1.23
 78 μl 153.5 ✓
 431.2 529.2 (use 98 μl / 100 μl)
 see p. 65

(5.4 R-XMS)
 23.7
 * 29 nmol nt/
 20.7 $\mu\text{g}/\mu\text{l}$
 65.6
 * see P55 f
 change to on
 of DNA plasm
 (50 μM each)

Reactions

	(1) 1-8	(2) 9-16	(3) 17-24	(4) 25-32	(5) 33-40
stop tube *					
<u>mix A</u>	98 μl				
TFI/Vent = 0.18 units (opimul TFI 5-14-91)	2				2 μl Epicenter storage buff
Vent lot #17 = (opened 2-24-95)					
0.09 u/ μl = 0.18 u		2			
0.5 = 1 u			2		
2 (no dilution) = 4 u				2	
V _f = 100 μl					

note: can
 to Vent also
 with gaps
 on P 20
 } dilute
 opimul
 TFI into
 buffer

68°C in 9600, remove 8 μl to 8 μl killing solution P.1
 spot 2 μl on PET and 10 μl on GFC.
 * note: 20 $\mu\text{g}/\mu\text{l}$ 23. mp19 is ~16% as much total DNA as
 gapped DNA in Tag unit assay (500 $\mu\text{g}/\mu\text{l}$) however
 m13 is almost all ssDNA substrate while gapped DNA
 may have ~20 ssDNA gaps.

at 5 10 20 40, 60, 70, 100, 120 min
 * spot DuPont on PET

From Page No. _____

Turnover (-284 pmol) percent turnover percent incorporation
 (100) $(\frac{\text{nmol incorporation}}{\text{nmol input DNA}})$

min

icenter	5	1	445.00	83	10.4	24	note: it app there is no T/O at c since T/O stops incorporation stops ~ 20 min
Tf1	10	2	1588.00	672	70.8	53	
vent	20	3	3904.00	1266	130.6	50	
	40	4	6787.00	3352	22.4	49	
	60	5	6818.00	3368	21.3	52	
	80	6	7009.00	3468	22.4	54	
	100	7	7002.00	3462	22.4	50	
	120	8	7164.00	3546	21.4	55	
	5	9	333.00	25	—	0	note: turnover low and ends in for Tf1/Vent. conclude most are not full. since low per incorp.
	10	10	350.00	54	—	0	
18 u	20	11	381.00	50	—	0	
Vent	40	12	521.00	122	37	0.9	
	60	13	832.00	282	43	1.6	
	80	14	1097.00	415	45	2.2	
	100	15	1474.00	615	45	2.7	
	120	16	1928.00	847	53	3.2	
	5	17	296.00	6	2	1.0	← about 25 max incorp 13.8% / 51 (51% seen at 120 min) 30 still more are not full
1 unit	10	18	508.00	115	16	2.6	
Vent	20	1	1322.00	535	25	6.8	
	40	2	3242.00	1524	52	13.8	
	60	3	6116.00	3006	42	17.6	
	80	4	8505.00	4238	45	22.1	
	100	5	11510.00	5788	52	22.9	
	120	6	13872.00	7004	52	27.3	
	5	7	750.00	—	—	—	more conclusions 1. T/O is occurring at an opt high rate (~20-22%) for Tf1, considering ~30:1 Tf1/Vent (in units) 2. it appears that substrates after 3' end accumulate during time. probably at hairpins (on MISS) rather than at full length. (since T/O stops shortly after incorporation stops even for 1
	10	8	1728.00	—	—	—	
4 units	20	9	4617.00	—	—	—	
Vent	40	10	9106.00	—	—	—	
	60	11	11531.00	—	—	—	
	80	12	12228.00	—	—	—	
	100	13	12432.00	—	—	—	
	120	14	11890.00	—	—	—	
	5	15	269.00	—	—	—	284 are AMP background
	10	16	278.00	—	—	—	
no	20	17	240.00	—	—	—	
enzyme	40	18	276.00	—	—	—	
	60	19	293.00	—	—	—	
	80	20	274.00	—	—	—	
	100	21	307.00	—	—	—	
	120	22	331.00	—	—	—	

3. Incorp plateau for Vent alone (~27% incorp) is lower than for Tf1/Vent (~55%)

With ss d & Und rst od by m

Date

7/7/95

Inv nt d by

R corded by
sawyer Pamb

Dat

7-1-55

T Pag N

Polars

Results of P64

Project No. C 23.7 n mol nt input ss M13 DNA
 Book No. Percent substrate copied
 67

age No.	incorporation	p mol	
23	632.00		
24	37364.00		
25	37879.00		
26	106114.00		
min	27	104061.00	
mply	28	34.00	
5	29	57217.00	57217
10	30	76607.00	76607
20	31	114434.00	11792
40	32	112213.00	11562
60	33	120505.00	12423
80	34	123172.00	12698
100	35	115839.00	11942
120	36	125999.00	12990
5	37	1441.00	96
20	38	600.00	(9)
40	39	1390.00	70
60	40	2498.00	205
80	41	4176.00	378
100	42	5526.00	517
120	43	6777.00	642
5	44	7820.00	753
20	45	2449.00	252
40	46	6061.00	624
60	47	15599.00	1602
80	48	31777.00	3276
100	49	40517.00	4177
120	50	50876.00	5245
5	51	52552.00	5412
20	52	62653.00	6459
40	53	36563.00	
60	54 #38	260.00	
80	55	83243.00	8582
100	56	58704.00	6052
120	57	82715.00	8527
5	58	73558.00	7585
20	59	69056.00	7119
40	60	65008.00	6701
60	61	502.00	
80	62	591.00	
100	63	450.00	
120	64	960.00	
5	65	307.00	
20	66	563.00	
40	67	463.00	
60	68	80862.00	
80	69	75044.00	
100	70	81620.00	

ave BKGD
= 5-12

ave
79175 ⇒ 194 CPM/pmol nt

To Page No. _____

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		R c rded by <i>[Signature]</i>	

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Project N

Exhibit L-124

m13 PCR system: more optimization of [primer 407], annealing temp. and [target]

B k N

Appl. No. 09/558,421

69

Page N

x A, enough for 28 runs: 28.0ul 10x PCR buffer ✓
 8.4ul 50mM MgCl₂ ✓
 5.6ul 10mM dNTPs ✓
 2094.4 ul H₂O ✓
 5.6ul T_{aq} 5u/ul ✓
 2520ul

template dilution:
 to 50pg = 1ul stock m13 RF
 370ug/mL
 + 99ul TE
 mix ✓
 3.1ul + 7.3ul TE ✓
 7.4ul of 50pg/ul

13 RF 3ul of 10pg/ul 9ul 10pg/ul 6ul of 50pg/ul ✓
 12ul 18 24 12.8ul 2.4 12.8 2.4 ✓
 15ul 9ul 3ul 3ul 0 12ul, 6ul, 0ul ✓

inner mix
 outer anchor
 outer 407
 anchor 407

x A 270ul → 270ul → 270ul →
 300ul →
 divide into 3 tubes, 1 for each annealing temp.
 a, b, c

a = 53°	10pg m13 RF	4 a 53° 30pg m13	7 a 53° 100pg m13
b 55°	✓ 400nm primers	b 55° 400nm primers	b 55° 400nm p
c 57°		c 57°	c 57°
a = 53°	10pg m13 RF	5 a 53° 30pg m13	8 a 53° 100pg m13
b 55°	✓ 400nm primer	b 55° 400nm p	b 55° 600nm p
c 57°		c 57°	c 57°
a 53°	12pg m13 RF	6 a 53° 30pg m13	9 a 53° 100pg m13
b 55°	✓ 800nm primers	b 55° 800nm p	b 55° 800nm primers
c 57°		c 57°	c 57°

To Page No.

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Recorded by

Date

7/5/95

70

Project No. _____

Book No. _____

TITLE _____

From Page No. _____

1% agarose gels:

6g agarose
600 mL 1x TAE

40ul 10mg/ml CtBr

M13RF: 10 pg

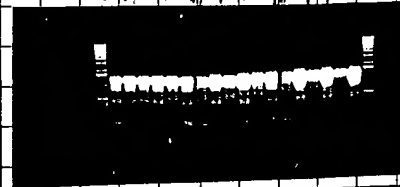
30 pg

100 pg

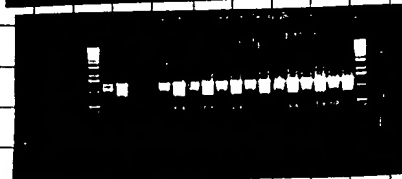
primer (nm) 400 400 800

cycles 30 25 2 2 2 2 2 2 2 2 2 2

top
57°C C 1-9
55°C B 1-9
53°C A 1-9
bottom



5



5



5

conclusion

1. no advantage to >400 nm primer
 2. more target improves yield and specificity
 3. 57°C is most specific
- Canton Combs
7/6/95

T Pag 1

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7/7/95

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[Signature]

Date

7/5/95

Tag No. _____

pg target

10

30

100

primer (nm)

400	600	800	400	600	800	400	600	800
130 55	130 55	130 55	130 55	130 55	130 55	130 55	130 55	130 55

cycle #

anneal
temp

57°C

55°C

55°C

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7-6-95

From Page No. _____

title: m13 PCR system: 4 annealing temperatures and 4 primer sets.

53°C, 55°C, 57°C, 59°C

 anchor + 6681
 7069
 407
 806

purpose: We have established optimal [template] & [primers] for anchor + 407. Now we'll optimize annealing temp and cycle ~~x~~ for the other primers 6681, 7069, 806. ~~we do it~~ assuming that these other primers will work well with the [template] & [primer] that worked well w/ 407. Later, we'll titrate [Taq] & [Tne] for these primers.

background: • 59°C has not been tried before w/ any primers

- in an earlier expt 57°C worked best for 407
- [template] = 100 pg / 100 µl rxn - found to be best for 407
- [primer] = 400 nM - found to be best for 407
- [Taq] = 1 U / 100 µl rxn, more was inhibitory for 407
2 U / 100 µl rxn will be tried for 806 which makes the longest product

• expected product sizes:

anchor + 6681 → 380 bp

+ 7069 → 768 bp

+ ⁴⁰⁷~~356~~ → 1356 bp

+ 806 → 1755 bp

Cauling 7/6.
Cmb

materials: m13RF 50 pg/µl, diluted on 7/6/95 NBH p.

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OBblays

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R c rded by

Paul m Pmb

Date 7/95

7/6/95

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procedure:

make a master mix for 21, 100ul rxns - containing everything but the primers:

(A) { 210ul 10x PCR buffer
1654.8ul H₂O
43ul 50mM MgCl₂ Cf = 1.5mM
42ul 10mM dNTP's Cf = 200uM
42ul m13mp19RF 50pg/ul stock
42ul anchor primer, 20uM Cf = 100pg/100ul rxn
stock Cf = 400nM
4.2ul Taq 5u/ul Cf = 1u/100ul rxn
2058ul

remove 441ul and add 0.9ul Taq (5u/ul) - for 806 primer
w/ 2u Taq/100ul rxn

is (1.5mL)	1	2	3	4	5
mer, 20uM stock	8ul 6681	8ul 7069	8ul 407	8ul 806	8ul 806
)	392ul				(B) →
	400ul				

divide into 4, 100ul aliquots in 9600 PCR tubes
and put each tube in different 9600's set to different
annealing temperatures 53° - Lab 15

55°C - Lab 16

57°C - SGT

Paula 7/6/95
Combs

59°C - Lab 14 * note that 30 cycle
aliquots were taken

during ramp to 94°C

Pause the 9600's during later part of the 70°C, 2'
extension to withdraw 25ul samples at 25, 30, 35 cycles -
+ 2.8ul Blue Juice

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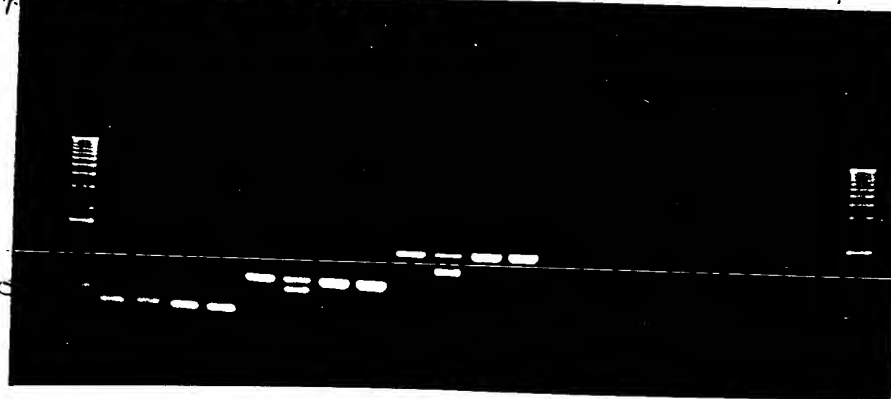
Date

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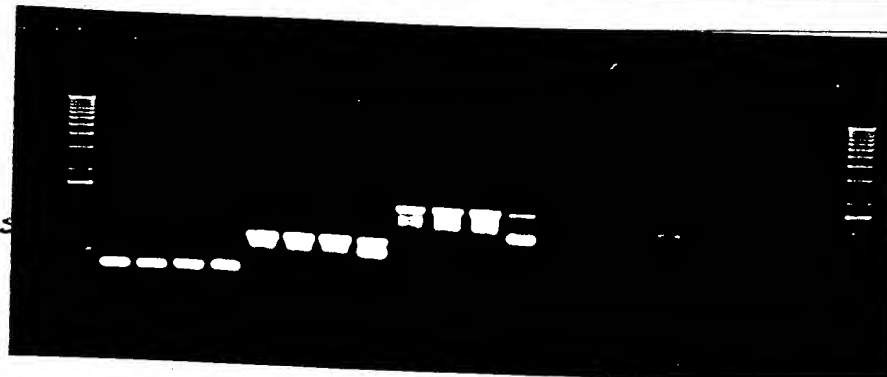
primer
annealing temp
6681 7069 407 806 806

100

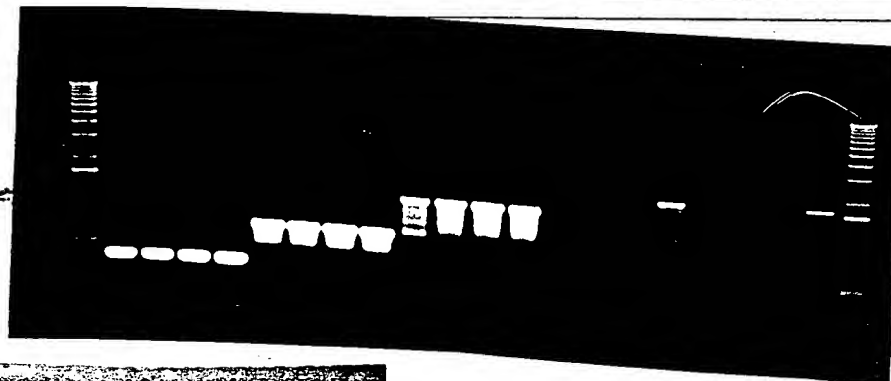
25
cycles



30
cycles

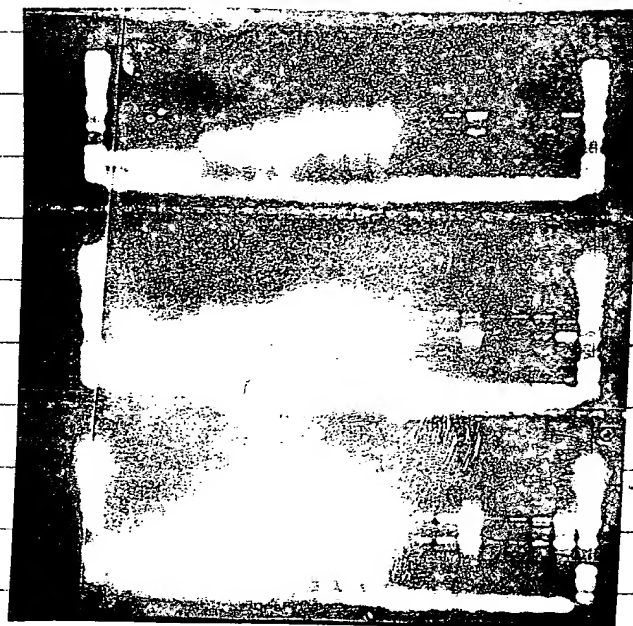


35
cycles



Note: 59°C
cycles - 1
9600 during
to 94°C so
product is,
ssDNA - acc
for the 2,
in 7069 & 407

Pauline
Combs
7/6/95



25 cycles

30
cycles

35 cycles

• 59°C annealing temp for
25 cycles gave a good product
yield and the least nonspecific
products for primers 6681,
7069, 407
• Try higher annealing temps for
primer 806 because product is
beginning to come up at 59°C

With ss d & Und rsto d by m ,

Pauline

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7/7/95

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R corded by
Pauline Combs

Date
7/7/95
7/6/95

m13 PCR system: titration of [Tne] and [Tag] with 6681, 7069, & 407 primers - 3 cycle x 5

age N _____

purpose: 1) To optimize the [Tag] - \rightarrow 100 μ l rxn was previously found to be inhibitory, so lower conc. will be tried - 0.25 μ l

2) To see if Tne can synthesize any of the products expected w/ 6681, 7069, & 407 primers + anchor primer - no product was made (p. 44) when 50 Tne/100 μ l was tried. The [template] and [primers] that were optimal for Tag will be used in the Tne PCR rxns. 0.1 - 1 μ l will be tried

- 25, 30, 35 cycle samples will be taken
- 59°C annealing temperature, 400 μ M primers, 100 pg/100 μ l rxn m13 target

sterilize:

mix [A] w/ 6681, for 14.5 rxns

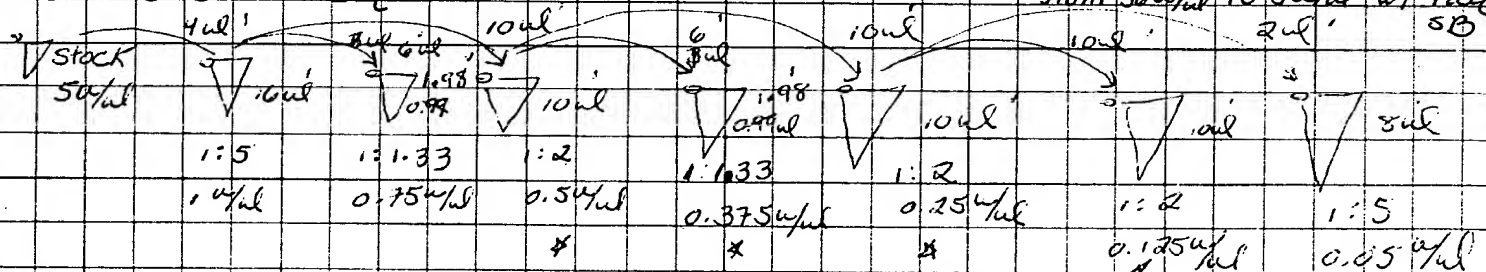
885.5 μ l H_2O	
115 μ l 10x PCR buffer	
34.5 μ l 50mM $MgCl_2$	Cf = 1.5 mM
23 μ l 10mM dNTPs	Cf = 200 μ M
23 μ l 20mM anchor	Cf = 400 nM
23 μ l 20mM 6681 primer	each
23 μ l m13RF 50 pg/ μ l	Cf = 100 pg/rxn
112.7 μ l	

see p. 69 for dilution of stock to 50 pg/ μ l lot FAS 701

mix [B] same as [A] but use 23 μ l, 20mM 7069 primer

mix [C] same as [A] but use 23 μ l, 20mM 407 primer

The dilutions in Tag SB: RT, rinse pipet, vortex 2 sec



Also do same dilutions w/ Tag in Tag SB - only 1 dil will be used

Step sold = 100mM EDTA \rightarrow 80 μ l 0.5M EDTA + needed to kill exo
 8x Blue Juice + 320 μ l 10x Blue Juice ice won't kill it
 400 μ l

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Date

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7/6/95

Emilia Corns

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76

Book No. _____

TITLE _____

Form Page No. _____

run #	1	2	3	4	5	6	7	8 → 14	15-21
	98ul [A]						1	98ul [B]	98ul [C]
1.4% Tne	2ul							same as 1-7	same as 1-7
0.75% Tne		2ul							
0.5% Tne			2ul						
0.375% Tne				2ul					
0.25% Tne					2ul				
0.125% Tne						2ul			
0.05% Tne							2ul		
	100ul								

* remove 25ul rxn after 25, 30, 35 cycles to 2.8ul Blue Juice

note: 59°C annealing temp, 10:40 AM - 2
 { 94°C 15" } Lab 15
 { 59°C 30" } program 74
 { 70°C 2' extension } 9600
 4°C final

run #	22	23	24	25	26-29	30-33
	98ul [A]				98ul [B]	98ul [C]
0.5% Tag	2ul				2	2
0.375% Tag		2ul			2	2
0.125% Tag			2ul		2	2
0.05% Tag				2ul	2	2

30 cycles only

** remove 25ul to 2.8ul Blue Juice

* remove 25ul of Tne rxns after 25, 30, 35 cycles during last part of 2min, 70°C elongation into 4ul stop soln in microtiter plate. The final [CDTA] = 10mM
 [Blue Juice] = 1.1X

** remove 25ul of Tag rxns after 30 cycles + 4ul stop soln

T Page N

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M Polamp

Date

7/7/95

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R c rd d by

Radm. Pombh

Date

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7/7/95

Polany

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Exhibit L-127

Book No. _____

Appl. No. 09/558,421

78

TITLE

GAPDH

pUC

From Page No. _____

10 R

5 x Chemo (no dNTPs)
(P2, 10)[A]
100[B]
100

10 mM dNTPs

10

10

(200 μ l)Human spleen genomic
DNA (HS #2 19/4)
80 ng/ μ l

12.5

(100 ng/5 μ l)GAPDH(+) 2112, 10 μ M

20

CF
(400 nm)GAPDH(-) 2113 10 μ M

20

Mg OAC 100 mM

—

2.5

{ CF mg
for
CF=1.
for BpUC DNA
Xmn I 25 μ g/ μ l

10

2836, 10 μ M

20

2837, 10 μ M

20

H₂O317.5
470317.5
470[A]
[B]

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

48 —————→

48 —————→

The 5-7-95 (Liz)

0.5

2

2

1.5

2

2

2

2

2

2.5

2

2.5

The 534 μ l Adam 3-2-95
delta 24 μ l

1 2

1 2

Tag 2 μ l

1 2

T Pag N

With ss d & Und rsto d by m ,

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7/14/95

Inv nt d by

R corded by

Dat

7-10-95

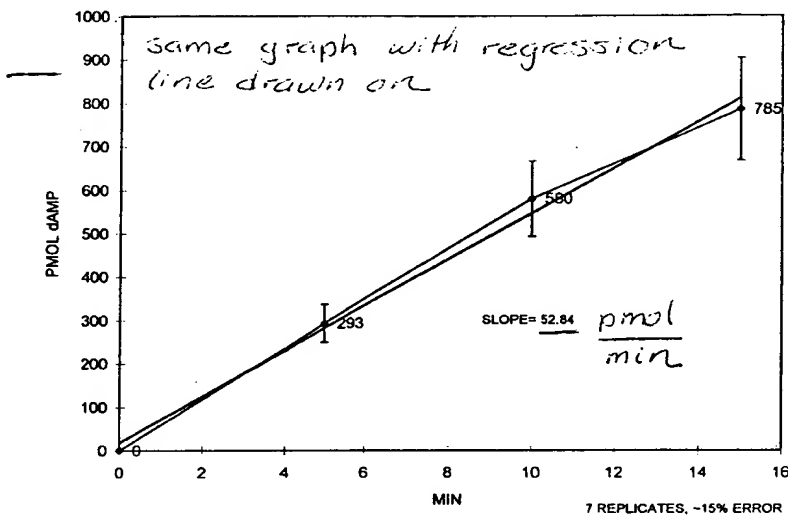
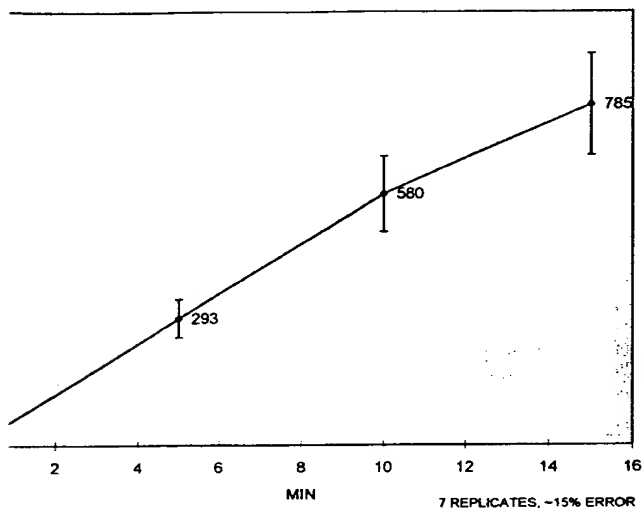
all dirty
made with
Tag stock
buffer

Sheet3 Chart 1

Sheet3 Chart 1

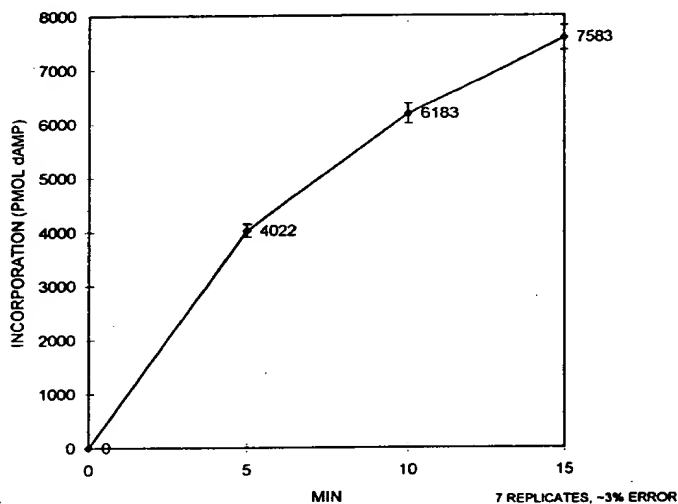
LT'S TivVent IN EPICENTER'S SB: TURNOVER AT TIME ZERO IN STABILITY STUDY (6/28/95)

LT'S TivVent IN EPICENTER'S SB: TURNOVER AT TIME ZERO IN STABILITY STUDY (6/28/95)



Sheet4 Chart 1

LT'S TivVent: POLYMERIZATION AT ZERO TIME POINT IN STABILITY STUDY (6/28/95)



calculations:

To Page No. _____

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Date

Recorded by

7/11/95

Carolyn Combs

M13 PCR: Titration of Tne & Tag in Cheng buffer

Project N

Book N

Exhibit L-129

Appl. No. 09/558,421

81

age No. — purpose: Can we make the 380bp m13 PCR product w/ Tne? Earlier p. 77 w Tne failed to make this product in 10x PCR buffer.

A	for ¹⁷ rxns, 100ul per rxn:	340	300ul	5x Cheng buffer
		34'	30ul	10mM dNTPs $C_f = 200\mu M$
		34	30ul	50 pg/ul m13mp19 RF [*] in TC $C_f = 100\mu g$
		34'	30ul	20uM anchor primer
		34	30ul	20uM 6681 primer $C_f = 400nM$
		1190'	ul	H ₂ O

1666 ul 1470 ul

ions of Taq & Tne (5/7/95 Lig) in Taq storage buffer (SB)

5	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
98ul A																		
2ul rTag								2ul Tne (5-7) 45Liz										
2								2										
			2								2							
				2								2						
					2								2					
						2								2				
							2								2			
								2								2		
									2								2	
100ul																		

- 15', 1 min 94°C
- 94°C 30" denat.
- 55°C annealing temp 30"
- 72°C exten 2'
- remove ~~20~~²⁵ul aliquots after 25, 30, 35 cycles
- program 76
- 16
13
18
- 1 pm - - 4 pm
- * remove 25ul p. 79
- + 3ul STOP soln (w EDTA)
- * run 25ul

To Page No.

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Polansky

Date
$$\frac{5}{114/95}$$
Invented by

Recorded by

Date

7/11/95

Project No. _____

Book No. _____

TITLE m13 PCR: 3 small products w/ Tne

84

From Page No. _____

purpose: To determine if Tne can make the 3 smallest m13 PCR products 380 bp, 768, 1356 bp, 380t using the conditions which worked for gapDH & PUC p80. (Cheng buffer, 400 nM p, 35 cycles, 55°C annealing temp & 94°C 30" denat, 100 pg/ul, 72°C 2' extension)

- If the products are made, we can use these conditions for m13 primer extension experiments

materials: mix for 15, 50ul rxns

[A] 150ul 5x Cheng
510ul H₂O
15ul 10mM dNTPs
15ul 50pg/ul m13mp19 RF
15ul 20uM anchor primer
705ul

[B] = 235ul A + 15ul 20uM 6681

[C] = 235ul A + 15ul 20uM 7069

[D] = 235ul A + 15ul 20uM 4071

dilutions of Tne (5/7/95 Lig) in Taq storage buffer:

50ul stock
10ul
+23.3ul TaqSB 33.3ul
1:3.33X
1.5uM

20ul
+10ul TaqSB 30ul
1:1.5
1uM

20ul
+20ul TaqSB 40ul
1:2
0.5uM

20ul
+20ul TaqSB 40ul
1:2
0.25uM

20ul
+20ul TaqSB 40ul
1:2
0.125uM

rxn 1 2 3 4 5 6 7 8 9 10 11 12 13

[B] 48 48 48ul
[C] 48ul
[D] 48ul

1.5uM Tne 2 2 2 2 2 2 2 2 2 2 2 2 2
1uM 2 2 2 2 2 2 2 2 2 2 2 2 2
0.5uM 2 2 2 2 2 2 2 2 2 2 2 2 2
0.25uM 2 2 2 2 2 2 2 2 2 2 2 2 2
0.125uM 2 2 2 2 2 2 2 2 2 2 2 2 2

→ start rxns on ice w/ en

Result on p. 86
Only the 380t product was made.

Witnessed & Underst d by m ,

[Signature]

Date

7/14/95

Inv nt d by

[Signature]

R c rd d by

Paulson Pomb

Dat

7/12/95

To Page N

³²P-23. M13 for TFI/vent

ig No. _____ same experiment as P. 64 except ³²P 23 instead of ³²PDA
2 increasing T/O with time (P. 64-68) due to strong pause site
visible on an agarose gel?

5 ng / λ 23mer
5x Kinase buffer
PNK
ATP 10 mg/ml 7-14-95

35 μ l
10.2 μ l
1.2 μ l
5

1.75 ng
25 pmol 23mer

51.4 μ l

37°C, 30 min \rightarrow 55°C, 5 min

51.4 μ l
42.6
10.6

H₂O

M13 mp19 0.26 μ g/ λ
0.79 nmol nt/ λ
0.109 pmol circle/ λ

11.5 pmol
circle tot

200

$\frac{23mer}{circle} = 2$

70°C, 5' cool slow

Cf = 0.41 nmol nt M13/ μ l

it will be ~23.7 total nmol nt/100 μ l Rxn
same as for P. 64

To Page No. _____

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Polansky

Date
7/14/95

Inv nted by
R corded by

Date
7-13-95
RZ

From Page No. _____

(A)

3.5 R₁₀₀32P23_{mem} - mPIA (P87)

171.1

0.41 nmol nt / μ l0.566 pmol acid / λ

5X Chumy (no dNTPs)

70

P21, 10

10 mM dNTPs

1.75

C_f = 5 μ mH₂O

100.15

V_f = 343(use 98 μ l / 100 μ l R₁)

①

②

③

(A)

9 μ l

2

Tf1/Vent
(equivalent Tf1)

5-16-95

(1.88 μ l total in
← its only 0.94
equivalent units) due
with Vent by Nir

Tf1 lot 31010A-502

1.88 μ l(1.88 unit H₂O)1 μ l (equivalent units)Vent 2 μ l

2

lot #17

opened 2-24-95

(4 units) at 1.88 is
more Vent than in Tf1,
in order to get full length
products)68°C. Remove 8 μ l at 1, 2, 5, 10, 15, 20, 40, 60, 90
to 1 μ l 10X 'blue juice' mM E.

Run on agarose (same as P56, 9)

T Page N

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D. Polansky

Dat

7/14/95

Invent d by

R c rded by

Dat

7-14-95

Project No. _____

Book No. _____

TITLE _____

Exhibit L-132

Appl. No. 09/558,421

From Page No. _____

1% agarose gel

tip ~~empty~~

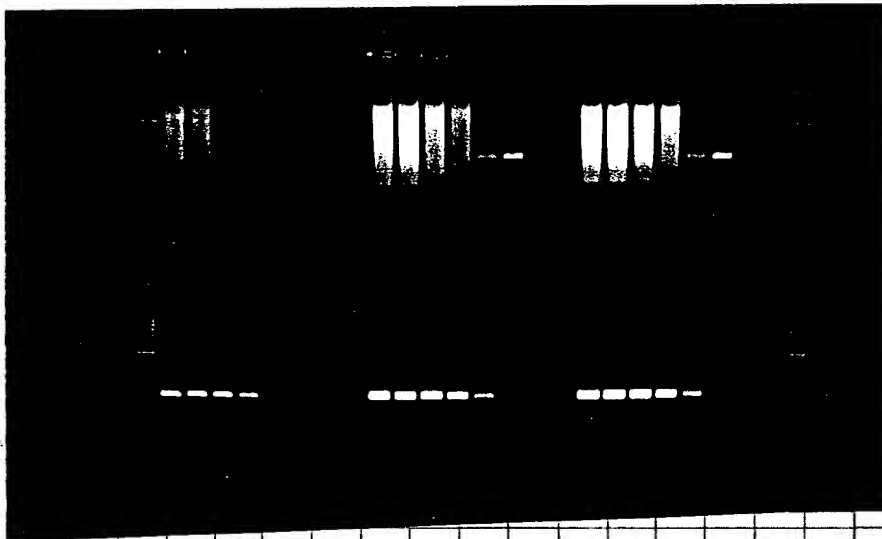
9-16	9-16	9-16
25	30	35

inc 9-16 decreasing eny

1-8	1-8	1-8
25	30	35

cycles Tag 1-8 decreasing eny - run into gel 1st

The from p. 80



same photo as p.
taken from a further
distance to capture
the ~~with~~ bottom of
gel

To Page N .

Witnessed & Understood by me,

Dat

7/14/95

Investigated by

Recorded by

Pamela P. Smith

Dat

7/14/95

Page No. _____

Cheng buffer, 380bp product expected

25 cycles

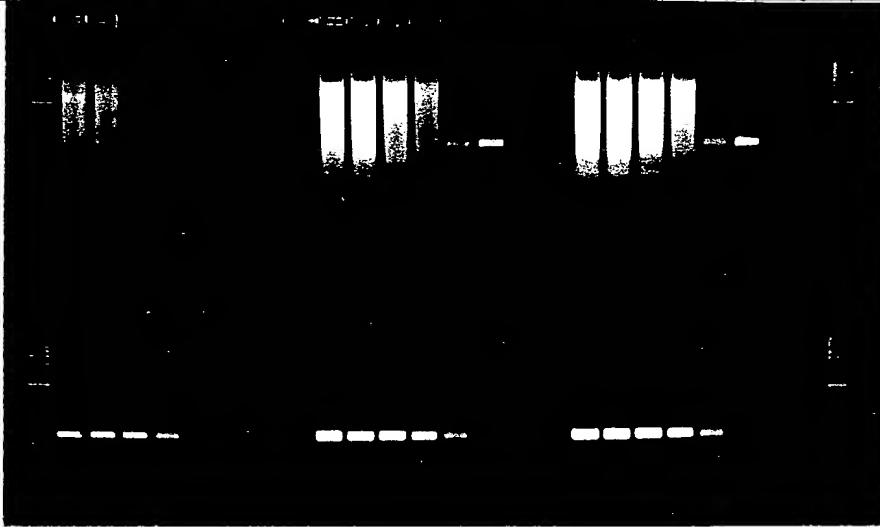
30 cycles

35 cycles

units

6 5 4 3 2 1 .5 .25 6 5 4 3 2 1 .5 .25 6 5 4 3 2 1 .5 .25

1,000ul rxns



Tne

rTaq

- 1 unit Tne is optimal, 35 cycles 21.5
- 6 units rTaq gave the most product for all cycle #3

To Page No. _____

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rPolamp

Date

7/14/95

Inv nt d by

Rec rd d by

Carlye Combs

Date

7/14/95

m13 PCR: A PCR buffer into Cheng
buffer

ag N — purpose: To find out what component(s) of Cheng
are important for making the broad
smear (vs narrow, low mwt smear made
w/ PCR buffer)

2.1: 3g agarose.
4 mL 50X TAE.
294 mL H₂O
300 mL wt 4.14.5g w/ magnet
+ 20 mL CHBr₃

reagents: [A] for 35 rxns = 35 μ L 20 mM anchor primer
35 μ L 20 mM 6681 primer
35 μ L 10 mM dNTPs
910 μ L H₂O
1015

[B] w/ target
507.5 μ L of [A]
+ 17.5 μ L 500g/mL
m13 RF

[C] no target DNA
507.5 μ L of [A]
+ 17.5 μ L H₂O

*Nens 2/10/95

[D] = mix of 200 mM Tricine + 10.05 mM MgOAc to add KOAc to = 10X working stock
200 μ L of 1M Tricine pH 9 (from Nens)
+ 10.05 μ L of 1M MgOAc
+ 789.95 μ L H₂O
1 mL

[E] = mix of 166.6 mM Tricine pH 9, 708 mM KOAc, 8.75 mM MgOAc
is 8.33X stock 166.6 μ L 1M Tricine pH 9
354 μ L 2M KOAc
8.75 μ L 1M MgOAc
470.65 μ L H₂O

Tne (5-7-95) Liz 5 μ L diluted to 0.2 μ L w/ Taq storage buffer + 148.8 μ L Tne
15 \rightarrow 28 \rightarrow 12/7/95

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
buffer	5																											
NgCl ₂	1.5																											
		5	5	5																								
1 KCl		10																										
1 KOAc			2.35	5.88																								
						6	6	6	6	6	6	6	6	6														
lycerol						2	5	8						8	8	8												
MSO	8.5	0	7.65	4.12						.5	.75	1	.5	.75	1													
Tne	5					9	7	4	8.5	8.5	8.25	8.25	0.5	.25	0													
	50 μ L																											

To Page No. _____

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Polamp

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7/14/95

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Date

7/14/95

From Page No. _____

Buffer components

Tris pH 8.4 20

KCl 50

MgCl₂ 1.5

Tricine pH 9

MgOAc

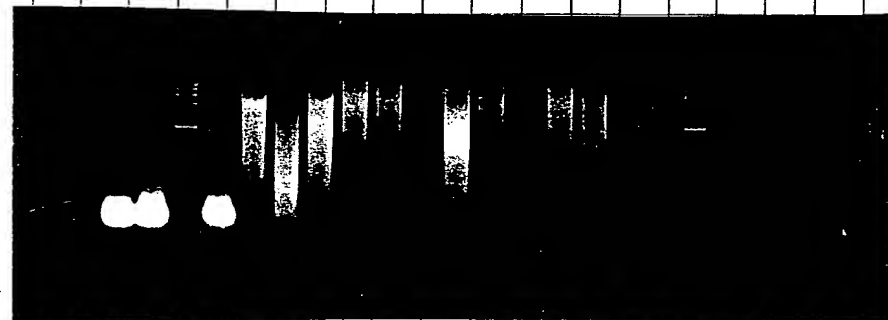
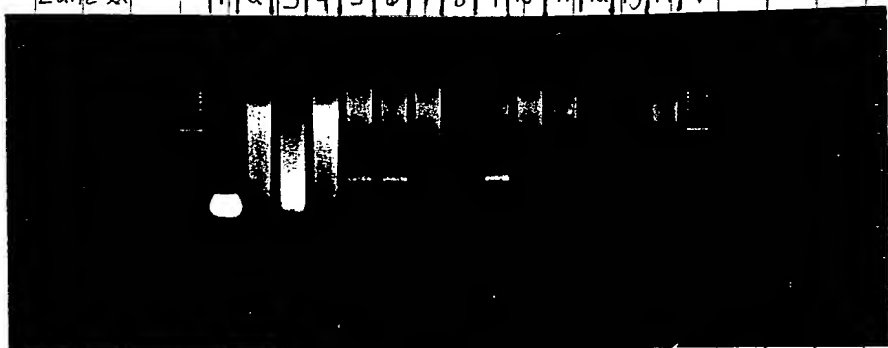
KOAc

glycerol

DMSO

Lane #

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Tris pH 8.4															
KCl															
MgCl ₂															
Tricine pH 9															
MgOAc															
KOAc															
glycerol															
DMSO															



anchor primer alone
anchor primer alone

- 1) The transition from "primer dimer" (smallest band) to smear occurred when Tricine pH 9 + MgOAc 1.5 mM was substituted for Tris pH 8.4 + MgCl₂
- 2) Product was made when 85 mM KOAc was added to the Tricine MgOAc buffer - glycerol + DMSO were not required to make product
- 3) Less glycerol + DMSO resulted in more product synthesis
- 4) The smears are present in the plus and minus target lanes
- 5) The "primer dimer" is made when either primer is present alone or if both primers are present as long as PCR buffer is used

With ssd & Understood by m,

S. Polamp

Date

7/14/95

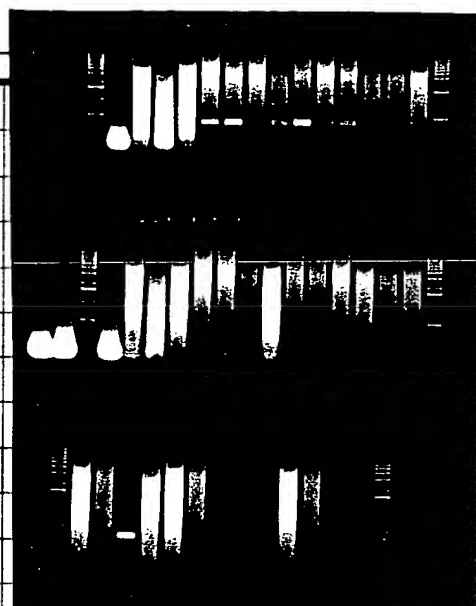
Invented by

R. Cord g by

R. Cord g by

Date

7/14/95



Expt on p. 87

+m13

template + 2 primers
for 380 bp product

*Note the enzyme
brings glycerol in
rxn, 50% glycerol
7, 10, 13%

-m13

template + 2 primers

no template, only

Project N _____

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TITLE _____

is 32 P primer incorporated into either
the "primer dimer" or smear acc.
The on P. 86 ?

From Page No. _____

	(- Target)	(+ Target)	(32 P M. KOAC)	
	(A)	(B)	(C)	(10 Rens /
1 M Tris pH 4	10			20 mM
3 M KCl	8.33	8.33	-	(50 mM)
50 mM MgCl ₂	15			(1.5 mM M)
1 M Tris pH 9		10	10	20 mM
MgOAC 50 mM		10.5	10.5	(1.05 mM)
M13 RF 50 µg/µl	-	10	10	(50 µg / 50 µl PCR
10 mM dNTPs	10	10	10	2 M KOAC
H ₂ O	366.7	361.2	350 348.25	
	VR = 410	410	-410	

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17

(A)

41 µl

(B)

41 µl

(C)

41

6681 10 µM	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
6681 10 µM			2		2		2		2		2		2		2	
301 10 µM	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
6301 10 µM	2			2			2			2			2			2

Cf = 400 nM

The 5-75 (Liz) 5 µl

0.2 µl/µl diluted

in Tag SB

35 cycles as per P. 86

note for 3 µl
80% PAGE ita
0.2 µl 32 P primer

mix 20 µl PCR with 2 µl BJT, 100 mM EDTA load 10 µl on 80% Ag
as per P. 56 9 150 V start ~ 2 PM (?)
Morus as per AK book 7 start 4:30

To remain 30 µl PCR add 15 µl cycle mix stop, load 5 µl
on 80% PAGE (wells 1-17 are PCR reactions)

for sequencing Rxn use 32 P 6681 - mp19 ss DNA (P. 71) as per
(P. 27, 4)

T Page 1

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S. Polans

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7-14-95

³²P 6681 and ³²P 6301

Project N _____
Book No. _____

91

g N		(can see P150, 9)	①	②		
100 μ M	6301 (anchor)	1.57			✓	Cf = 10 μ M Cf
100 μ M	6681		1.57		✓	
32 P γ ATP	10 mCi/ μ l	10	10		✓	
2 \times 7-1495						
1	PNK 1 μ l	1	1		✓	
5 \times	Kinase buffer	3.1	3.1		✓	Cf at 1X Kinase buffer
		15.66 μ l	15.6			35 mM Tris pH 7.6
						50 mM KCl
						5 mM MgCl ₂
0 μ M	6301 cold	78.3				
μ M	6681 cold	93.98	78.3			

is dilute hot primer with 5 parts cold primer
so contribution to PCR of P90 is:
primer in PCR

MgCl ₂	0.83	0.033
Tris pH 7.6	5.8 mM	0.23 mM
KCl	8.3	0.33

³²P 6681 = mp19 for sequence

mp19 0.26 μ g/ μ l 0.109 pmol ends/ μ l 10 μ l (1.09 pmol ends)

³²P 6681 10 μ M primer 1 10 pmol primer
70°C cool slow

for 3 μ l on PAGE is ~0.04 μ l primer

To Page No. _____

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Date

7-16-95

From Page

1.5 mM

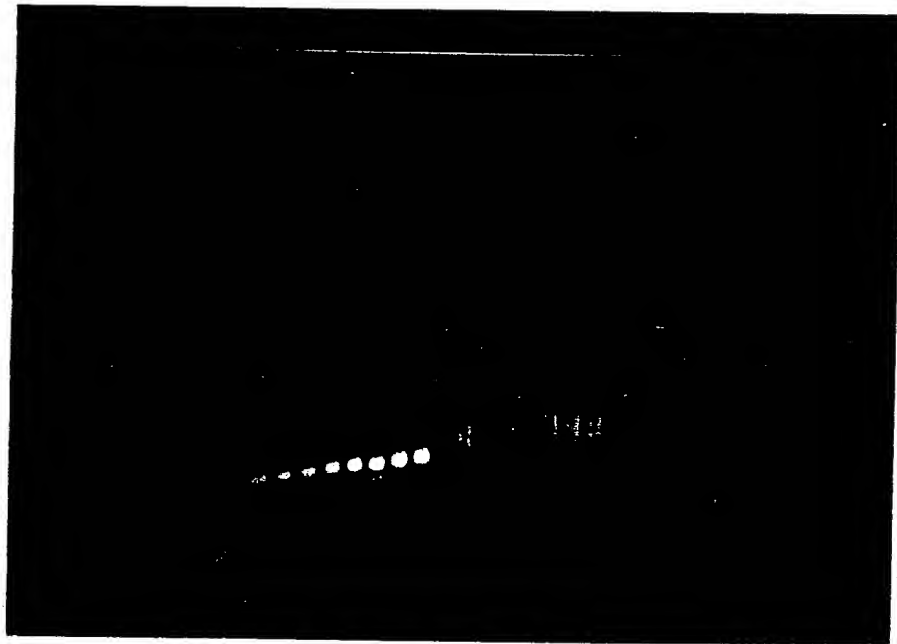
MgCl₂

1.05

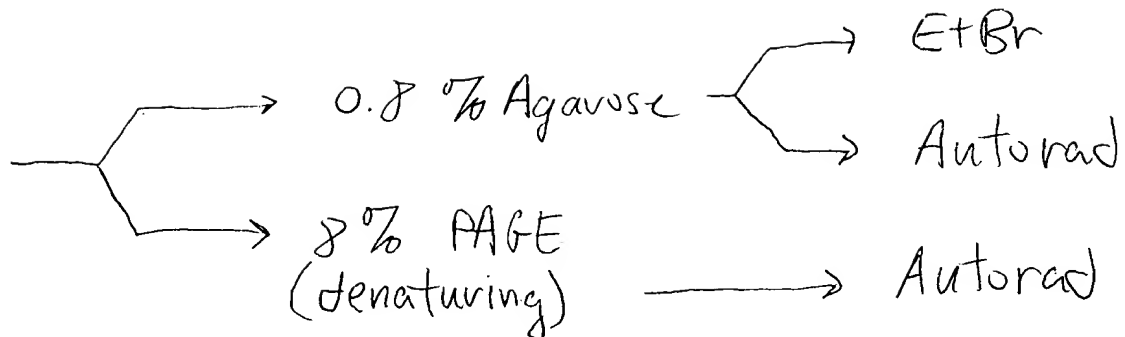
MgOAc

Tris
pH 8.4Tricine
pH 9

KOAc 85 mM



PCR
Tne
32P primers



With ss d & Und rst d by m ,

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Dat

R c rd d by

7-14-97

PAGE 93 OF NOTEBOOK WAS BLANK

From Page No. — 7/14/95 purpose: To determine which buffer component(s) were resp

1) for the transition seen from "primer dimer" to "smear" on p. 86 lab

The "primer-dimer" condition was 20mM Tris 8.4

50mM KCl

1.5mM MgCl₂what comp
is causing
transition

The "smear" condition was

20mM Tricine pH 9

50mM KCl

1.05mM MgOAc

{ Tris 8.4 vs
[Mg]
MgOAc2) Is the "smear" or "primer dimer" due to DNA contamination of the prep? materials:180 μ l 0.2 μ l/l Tris (5-7-95 Liq) \rightarrow 7.2 μ l The stock 5 μ l/l
+ 172.8 μ l Taq storage buffer

100mM Tris 8.5 * note this is a change from the original 8.4 "dimer" condition

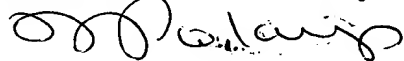
100 μ l 1M Tris 8.5 (RL)900 μ l H₂O

100mM Tricine pH 9

100 μ l 1M Tricine pH 9 (NEN 2/10/95)900 μ l H₂O5.25
10.5mM MgCl₂210 μ l 50mM MgCl₂ - 105 μ l
790 μ l H₂O - 895 μ l7.5
15mM MgCl₂300 μ l 50mM MgCl₂ - 150 μ l
700 μ l H₂O - 850 μ l5.25
10.5mM MgOAc10.5 μ l 1M MgOAc vs 5.25 - 10.5 μ l 5.25
989.5 μ l H₂O 1989.5 μ l 994.7
2mL7.5
15mM MgOAc15 μ l 1M MgOAc - 15 μ l 7.5 μ l
985 μ l H₂O - 1985 μ l 992.5 μ l
2mL

T Pag N

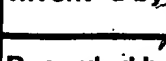
Witnessed & Und rst d by me,



Dat

7/14/95

Invent d by



R c rd d by

R. C. R. Davis

Dat

7/14/95

g N .—

res:

A = 30ul 10mM dNTPs ✓
 29.88ul 3M KCl ✓
 726.12ul H₂O (1st) ✓
 792ul

[B] template + primers

374ul [A] ✓
 17ul 20uM anchor primer ✓
 17ul 20uM 6681 primer ✓
 17ul 50 pg/ul m13RF ✓

425ul

use 25ul per 50ul rxn → This will give

t up each rxn in duplicate

er components:

		small smear condition = PCR buffer	tricine instead of Tris	1.05 Mg ²⁺ instead of 1.5	MgOAc instead of MgCl ₂	change [Mg ²⁺] standard	broad smear condition	Tricine w/ high [Mg ²⁺]	Tricine w/ MgCl ₂
# ↓	rxn #	1,2	3,4	5,6	7,8	9,10	11,12	13,14	15,16
Tris pH 8.4		✓		✓	✓	✓			
KCl		✓	✓	✓	✓	✓		✓	✓
n MgCl ₂		✓	✓						
Tricine pH 9			✓				✓	✓	✓
uM MgCl ₂				✓					✓
n MgOAc					✓			✓	
nM MgOAc						✓	✓		

To Page No. _____

ed & Understood by me,

D. Kemp

Date

7/24/95

Invented by

R. Corded by
C. Corded by

Dat

7/14/95

From Page N .__

[illegible]

With ss d & Und rstood by me,

Dat

Inv nt d by

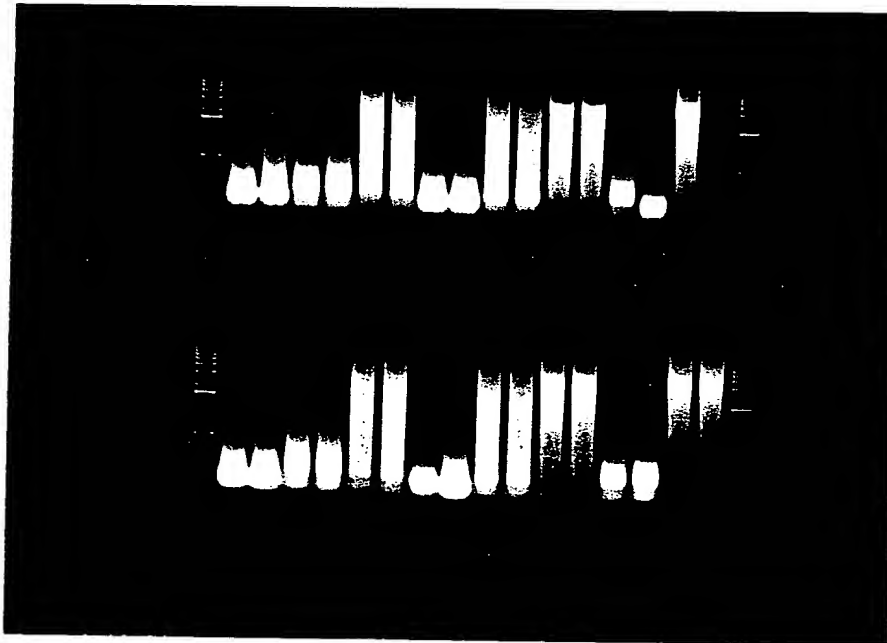
Dat

R e c o r d e d b y

T Pag

ag N _____

> Tris w/ 1.5 mM $MgCl_2$
 > Tricine w/ 1.5 mM $MgCl_2$
 > Tris w/ 1.05 mM $MgCl_2$
 > Tris w/ 1.05 mM $MgOAc$
 > Tris w/ 1.05 mM $MgOAc$
 > Tricine w/ 1.05 mM $MgOAc$
 > Tricine w/ 1.5 mM $MgOAc$
 > Tricine w/ 1.05 mM $MgCl_2$



} template + primers
 } no DNA
 } ie no template
 } no primers

ecomb

5/14/95 CC

all at 50 mM KCl

lower concentration of Mg^{2+} in Cheng vs PCR buffer is responsible for the broader smear. The Tris/tricine pH difference and $MgCl_2$ vs $MgOAc$ do not affect the transition from small^{narrow} to broad smear.

its model: distance between primers
 low $[Mg]$
 high $[KCl]$
 low $[Tne]$

short = small narrow smear
 long = broad smear

✓ by decreasing primer annealing
 ✓ by inhibiting Tne from binding primers

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Date

Recorded by

20 camp

7/21/95

Envelop Enrich

7/14/95

N _____

15 9600 - 94°C 1min
 94°C 30 sec
 - 55°C 30 sec } 35 cycles
 72°C 2min
 4°C hold

rxns by adding 7.5 ul of Stop soln = 8x Blue Juice Cf = 1x
 100mM CDTA Cf = 12.5mM CDTA

Stop soln made for future use = 50% glycerol
 100mM CDTA (10x)
 0.6x TAE
 BøB

for 5mL: 2.5mL 100% glycerol
 1mL 0.5M CDTA
 1.5mL 1x TAE = 30ul 50x TAE stock + 1470ul H₂O
 + pinch of BøB

To Page No. _____

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Polay

Date

7/14/95

Invented by

Recorded by

C. L. L. L.

Date

7/15/95

Buffers

From Page No. _____

Buffer C, 1 L ref NB 9, pg 162

✓ 17.1 mL 1M K phos monobasic
 ✓ 7.9 mL 1M K phos dibasic
 ✓ 80 mL glycerol
 ✓ 149.12 g KCl CF = 2 mL
 ✓ 0.2 mL 0.5M CDTA
 ✓ 350 µl 14.3M BMC - add
 ✓ qs to 1 L w/ H₂O

1M K phos monobasic
 68.045 g
 qs to 500 mL
 1M K phos dibasic
 114.115 g
 qs to 500 mL

+ 1 mL of 50% Tween 20 + NP40 - post filter det 1 mL + BMC

Buffer D, 8 L ref NB 9 p 182

✓ 200 mL 1M Tris 7.5
 ✓ 1.6 mL 0.5M CDTA
 ✓ 640 mL glycerol
 ✓ 2.8 mL 14.3M BMC - add
 ✓ 29.8 g KCl CF = 50 mL
 qs to 8 L

make 0.01% NP40 + Tween 20 for dialysis
 + 0.05% for Heparin column - filter 1 L + add 1 mL 5% NP40 + Tween 20 + 350 µl

2 L D + 700 µl
 400 µl 50% N

Buffer C, 500 mL ref NB 9 p 182

✓ 12.5 mL 1M Tris 7.5
 ✓ 0.1 mL 0.5M CDTA
 ✓ 40 mL glycerol add
 ✓ 0.175 mL β-me
 ✓ 174.5 g KCl CF = 2 mL
 ✓ qs to 500 mL

det 0.05% final
 1 mL/L

+ 5 mL 50% Tween 20 + NP40 after filtering

T Pag N

Witn ss d & Und rst od by m,

Dolamp

Dat

7/18/15

Inv nt d by

R c rd d by

Dandem Romh

Dat

7.18.15

small scale ext
and AmSO_4 (can see P163, 9)

Project N _____

Book No. _____

101

ig N _____ 7/18/95

20ml Tag extract buffer (P.167, 3) with 0.05% each NP40 / Tween 20
250ul 10mM PMSF

17.6ul 14.3M β ME

25ul Tween 20 \rightarrow 50% mix of both \rightarrow 5ml each

25ul NP40

4g cells -70°C Thorton shelf, Lab 16 - chip off 9503-15-764-D1-001K

10ml Tag extract buffer mix w/ spat in 50ml

20ml 0.2g cells/ml + 10ml pipet Falcon

cate tune - on XL2020 \rightarrow (0 turn, turn small to min
to tune) then min 1-5 minimize, not over 70
(stop)

9x 30sec pulses in ice- H_2O bath, ~1min between
pulses - should turn browner

75°C in Falcon - H_2O bath, then cool in ice- H_2O

Ke $\frac{\text{CF}}{200\text{mM}}$ NaCl + 5% PEI to CF = 1.5% \rightarrow 16mL vol extract in grad
58.44g/m cylinder

$$.2 \frac{\text{mole}}{\text{L}} \times .016 \text{ L} = 0.0032 \text{ M}$$

30mL centrifuge tube

$$\frac{\times g}{58.44 \text{ g/m}} = 0.0032 \text{ M}$$

0.187g NaCl ✓

1.45mL 10.8mL ✓
5% 16mL of 5% PEI while extract is
stirring in c. tube, 1 drop/sec

r 15min, 4°C

in 15min, 15K 5534, 4°C (~2700xg) DNA, cell debris & heat-denatured
proteins will ppt

cant into 25mL grad cylinder;

up = FI' fraction

nd AmSO_4 to help it go into soln

13.25 mL of FI' - .2 mL = 13.05 mL

\Rightarrow 2.297g ammon. salt

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Date

7/24/95

Inv nted by

[Signature]

Date

7-18-95

R corded by

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Book No. _____

TITLE _____

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From Page No. _____		remove 200ul aliquots of each fraction - eppendorf on ice add AmSO ₄ , spin 15 min, spin down 15K. 15; remove aliquot remeasure volume		
S30	AmSO ₄	vol (ml)	g AmSO ₄	slowly stir 15'
Fr I'	0	13.05	2.297g	
S1	30%	13.8	0.414g	
S2	35	13.5	0.182g	wrong 0.223g more added
S3	40-40	13.5 13.75	0.405g	0.223g 0.426g aliquot had x
S4	45	13.5	0.419	
S5	50	13.5	0.432	
S6	55	13.4	0.442	
S7	60	13.2		
S8	65			
S9	70			

mix: 150ul 0.5M Tapes 9.3 x 2 = 300ul 0.5M Tapes 9.3

A } 6ul 1M MgCl₂
 { 50ul 3M KCl

12ul 1M MgCl₂
 100ul 3M KCl

made 2

3mL

206ul mix A

6.3ul ³²P dCTP 10mCi/mL

60ul 10mM dNTPs

405ul 3.7mg/mL gap activated DNA

2.523mL H₂O

3.2mL for 6.7 runs with, use 48ul/rxn

6.4mL

412ul mix A

12.6ul ³²P dCTP 10mCi/mL

120ul 10mM dNTPs

810ul 3.7mg activated DNA

5.046mL H₂O

6.4mL

1:100 in Tag dilution buffer

52ul dil + 48ul reaction mix

10', 74°C

stop w/ 10ul 0.5M EDTA

spot 20ul on GFC filters

2ul aliquot + 198ul Tag dil bu

50ul rxn

+ 10ul stop

spot 20ul

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$$\left(\frac{\text{cpm}}{\text{specific activity}} \right) \left(\frac{\text{cpm}}{\text{cpm}} \right) = \text{p mole}$$

 VM CPM
 19195 c. comb

		pmol	units/ul
1 no eny	71.00	7.55	
2 FI	4679.00	498	7.47
3 SI	5411.00	576	
4 S2	5860.00	623	
5 S3	5434.00	578	
6 S4	3558.00	377	
7 S5	1394.00	148	
8 S6	299.00	31.8	→ 55% ammonium sulfate
9 mix	45046.00	} 2ul	$\bar{x} = 45048$
0 mix	44957.00		
1 mix	45141.00		

$$\text{specific activity} = \left(\frac{45048 \text{ cpm}}{2 \text{ ul}} \right) \left(\frac{50 \text{ ul rxn}}{2 \text{ ul}} \right) = \frac{28.2 \text{ cpm}}{\text{p mole nt}}$$

$$\frac{20,000 \text{ } \cancel{5000} (4) \text{ p mole nt}}{10,000 \text{ p mole dCTP}}$$

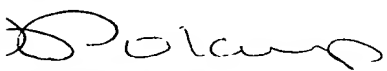
$$\frac{498 \text{ p mole}}{10 \text{ n mole}} \times 3 = \frac{\text{units (200)}}{2} = 7.47 \frac{\text{units}}{\text{ul}} \text{ in FI'}$$

$$100,000 \text{ units} / 3.5 \text{ g cells} = 28,000 \text{ u/g}$$

Lit - 22,000 u/g

To Page No. _____

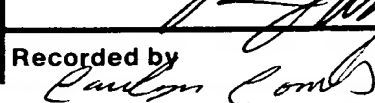
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7/24/85

Invented by



Recorded by

Paulson Comb

Date

7.11.85

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Large scale (81.5 g cells)

The prep (can see P176, 9)

Project No. _____

Book No. _____

105

ig N — except use detergent in Tag ext buffer

81.5 g

9503 -15-714 D1-001R The 5 vol

326 ml

Tag ext buffer (P167), 3: with
(Brown Temp) 0.05% each
Tween 20 / NP40

(Cf \approx 0.2 g / ml cells)

stir cells in RT buffer in beaker
strain thru cheesecloth

Mininganlin

10,000 PSI

1 pass

1/2 mL = FRI The, spun down cells 15 min 4°C - sup = FRI The

75°C 15 min (total time after Temp reaches 75°C)
cool fast in ice ~~shaking~~ shaking

vol = 405 ml

NaCl added to Cf 200 mM

= 4.73 g ✓

$$\frac{200 \text{ mmole}}{\text{L}} \times 0.405 \text{ L} = 81 \text{ mmole} \\ = 0.081 \text{ mole} \\ \times \frac{58.44 \text{ g}}{\text{mole}} = 4.73 \text{ g}$$

PEI for Cf = 0.5% add

45 ml 5% PEI pH 7.4 ✓ add

add dropwise, stir 15' more ✓

spin GSA 18,000 RPM 30 min ✓

ammonium & divide in 2

2 bottles

Sup = Fr I' / PEI

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vol of FRI'/PEI sup = 355 mL
 removed 1/2 mL = FRI'/PEI

$\frac{351g}{1000mL} = \frac{124.6g}{355mL}$ + ref R. Scopes. Protein Purification p. 304

add 124.605 g ammonium sulfate (ground up) for 55% sat
 added slowly over ~ 15 min while stirring at 4°

stirred 15 min, 4°C

spin down pellet 13K, 30 min GSA RC-5B, 4°C → pellet contains
 (spin in two bottles to produce 2 pellets of equal size) The DNA polymerase
 saved sup at 4°C in case activity didn't come down
 also 1/2 mL aliquot

respun 5K 5 min to pull as much liquid off the pellets as possible
 put the 2 pellets at -70°C

FPLC method for S200 column
 method 5 bank 2

METHOD 5 BANK 2
 0.00 CONC % B 0
 0.00 CONC % B 0
 0.00 FL/min 1.5
 0.00 PORT. SET 6.1
 0.00 PORT. SET 6.1
 0.00 UP. 1.00 1
 0.00 UP. 1.00 2
 0.00 CONC % B 0
 0.00 FL/min 1.5

0 conc % B 0.0
 0 conc % B 0.0
 0 mL/min 1.5
 0 port. set 6.1
 0 port. set 6.1
 0 valve pos 1.1
 0 valve pos 2.1
 400 conc % B 0.0
 400 mL/min 1.5

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Equilibrate 10ml supernatant
and S200

Project N _____
Book N _____

S200
column

107

ge N - wash with H₂O, (HiTrap Pharmacia)
equilibrate a 10ml blue column (used once and stored in
20% EtOH P1919)
at 3 col vol/hr = 0.5 ml/min
for 2 1/2 hr (= 7 col vol) with buffer B

wash S200 with H₂O (180 ml vol P178,9)
Equilibrate with buffer B
at 2 col vol/hr
= 90 ml/hr
= 1.5 ml/min } for S200 col will equilibrate
at 0.6 ml/min
= 500 ml in 14 hr (= 3 col vol)

S200 column

Resuspend one of the two AmSO₄ pellets of P.106
(i.e. 0.5 of the total material from 81.5g cells on P105)
in buffer B (~~and~~ containing detergent) (as per P178, 9)

final vol = 0.89 ml spun out insoluble material
in microfuge 15'
remove 20 ul to assay later AmSO₄ resuspend

Load by gravity on 180 ml S200.

elute at 1/2 col vol/hr (i.e. 1.5 ml/min) in buffer B

spin = 2A, 2 min/min, 1.5 ml/min, 3 ml/frn 2 min/frn

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7/24/91

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Chadler Comb

Date

7-19-91

7-20-91

From Page No. _____

unit assay on fractions 3-17 from S200 column

2ul fraction + 0.25ul Tag dil buffer \Rightarrow 1:31.622ul + 0.25ul Tag dil buffer \Rightarrow 1:31.62 total of 1:1000 dil

2ul of 1/1000 dil + 48ul mix A p. 102

+ a no eng control

10min 74°C - temp varied between 72-77°C

10ul STOP 0.5M CDTA

20ul on GPC - wash 1x 10%, 3x 5% TCA, 2x EtOH

dry 30min, count

specific activity = $\frac{\text{cpm}}{\text{SAM}} \left(\frac{60\text{ul}}{20\text{ul}} \right) = 28.2 \text{ cpm/pmol}$

SAM	CPM1	pmol		unit
1 no eng	41.00	4.36	546 were cloudy	no eng
fraction 23	52.00	5.53		3
from 34	51.00	5.43		4
S200 45	45.00	4.79		5
56	153.00	16.3		6
67	721.00	76.7		7 34/5
78	634.00	67.4		8 30
89	2027.00	216	prol fr 9, 10 = 8 ml total	9 97/2
910	4597.00	489	\hookrightarrow = 317,250 units loaded	10 220
1011	301.00	32	onto Blue Sepharose	11 4
1112	344.00	36.6		12
1213	820.00	87.2		13
1314	208.00	22.1		14
1415	531.00	56.4		15
1516	321.00	34.1		16
1617	254.00	27.0		17

unit def: $\frac{\text{unit}}{\text{ul}} \rightarrow \text{inc. } 10,000 \text{ pmole nt in } 30\text{min} \Rightarrow 2027 \cdot \frac{216 \text{ pmol}}{2\text{ul}} \cdot \frac{1000 \text{ ul}}{108 \text{ pmol}} = 1.08$

$1.08 \times 10^5 \frac{\text{pmol}}{\text{ul}} \left(\frac{30\text{min}}{10\text{min}} \right) = 3.24 \times 10^5 \frac{\text{pmole}}{\text{ul}}$

$\frac{10,000 \text{ pmole}}{\text{unit}} = 32.4 \text{ ul/ul} \times 3000 = 97,200 \text{ units}$

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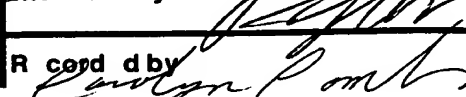
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gg N —
Blue Sepharose

load fr 9, 10 off S200 (P10P) $V_f = 6 \text{ ml}$
 $1 \text{ col vol/hr} = 10 \text{ ml/hr} = 0.17 \text{ ml/min}$

Wash O/N

7 col vol buffer B total = 70 ml

($0.08 \text{ ml/min} \times 70 \text{ ml in } 15 \text{ hr}$)

collect $10 \times 7 \text{ ml fractions of wash}$
 7 min/fr

THOD 5 BANK 2

0.00	CONC %B	0.0
0.00	CONC %B	0.0
0.00	ML/MIN	0.08
0.00	PORT. SET	6.1
0.00	PORT. SET	6.1
0.00	VALUE. POS	1.1
0.00	VALUE. POS	2.1
0.00	CONC %B	0.0
0.00	ML/MIN	0.08

FAIL. M=5 B=2
 11.31 RE= 11.31

C. Combs
 7/21/95

gradient : with scale down gradient
 for TPI of P 182, 9 &

for 10 ml Blue col

200 ml total gradient (20 col vol)
 of 50 mM - 1 M KCl

$3 \text{ col vol/hr} = 0.5 \text{ ml/min}$

3 ml/fr

6 min/fr

1 mm/min

start ~ 7:45 AM

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39 N
Blue Sepharose

load fr 9, 10 of S200 (P10P) $V_f = 6 \text{ ml}$
 $1 \text{ col vol/hr} = 10 \text{ ml/hr} = 0.17 \text{ ml/min}$

Wash O/W

7 col vol buffer B total = 70 ml
 $(0.08 \text{ ml/min} \times 70 \text{ ml in } 15 \text{ hr})$

collect 10 x 7 ml fractions of wash
 7 min/fr

THOD 5 BANK 2
 0.00 CONC %B 0.0
 0.00 CONC %B 0.0
 0.00 ML/MIN 0.08
 0.00 PORT.SET 6.1
 0.00 PORT.SET 6.1
 0.00 VALUE.POS 1.1
 0.00 VALUE.POS 2.1
 0.00 CONC %B 0.0
 0.00 ML/MIN 0.08

PRIL. M=5 B=2
 11.31 RE= 11.31

C. Combs
 7/21/95

gradient: will scale down gradient
 for TPI of P182, 9 &

for 10 ml Blue col

200 ml total gradient (20 col vol)
 of 50 mM - 1 M KCl

3 col vol/hr = 0.5 ml/min

3 ml/fr

6 min/fr

1 mm/min

start ~ 7:45 AM

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From Page No. — 7/21 Unit assay

 ^{32}P α dCTP ref 7/14/95

dilutions in Tag dilution buffer

• Fr T' / PET
355 mL

stock

Df = 1:100

2 μ l
+ 198 μ l
Tag dil buffer+ 40 μ l

1:2

Df = 1:200

+ 40 μ l

1:2

Df = 1:400

(1)

(2)

(3)

• Am SO₄ pellet
0.89 mL
resuspension

stock

1:100

2 μ l
+ 198 μ l

1:50

Df = 1:5000

2 μ l
+ 98 μ l40 μ l

1:2

Df = 1:10,000

40 μ l

1:2

Df = 1:20,000

(4)

(5)

(6)

• S200 pooled peak
fractions 6 mL

stock

1:10

2 μ l
+ 18 μ l

Df = 1:500

2 μ l
+ 98 μ l

1:50

40 μ l

1:2

Df = 1:1000

40 μ l

1:2

Df = 1:2000

(7)

(8)

(9)

• Fractions from Blue
Sephacose columnsundiluted wash fractions

W5 (10)

W10 (11)

F5 (12)

peak elution fractions 10-20

stock

1:10

2 μ l
+ 18 μ l2 μ l
+ 28 μ l

1:15

Df = 1:150

tube

(13) - (23)

Start runs by adding 2 μ l of each dilution to 48 μ l A mix p. 102

74 °C 10 min

+ 10 μ l 0.5 M EDTA, wash & spot 20 μ l

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7/21/95

R c rd d by

7/21/95

Pamela Pomb

ig N .

CFM1.

$$\frac{\text{pmole}}{\text{cpm}} = \text{specific activity}$$

1/100	4504.00
1/120	3334.00
1/400	1539.00
1/500	11945.00
1/6,000	8088.00
1/6000	4497.00
1/500	9437.00
1/1000	5329.00
1/2000	3011.00
wash 5	108.00
wash 16	98.00
fr 5	107.00
10	651.00
11	813.00
12	1642.00
13	3668.00
14	7866.00
15	10929.00
16	6668.00
17	6788.00
18	5668.00
19	3724.00
20	2935.00

1. from 13-17
we 2ml (off 3 ml) from each fraction $\therefore V_p = 10 \text{ ml}$

Analysis of blue pool

Analyse against 1800 ml buffer D (pH 8.0)

continued on p. 114

To Page No._____

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7/21/97

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Recorded by

Recorded by
Carolyn Court

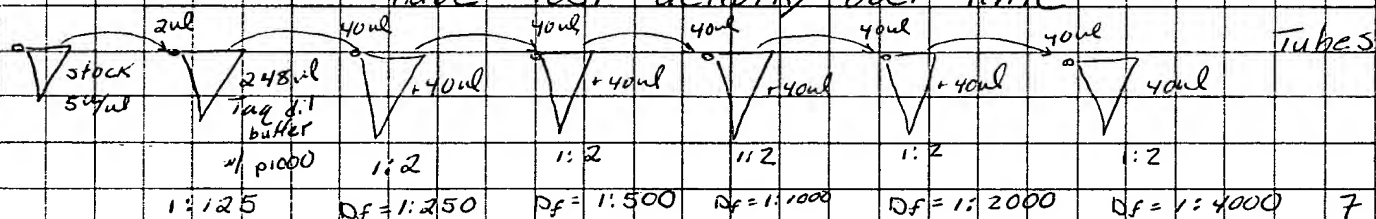
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From Page No. — Unit assay on old Tne prep (5-7-95) side-by-side the new Tne pool which was eluted from Blue agarose column on 7/21/95 p.111 NB11. Sepharose

enzyme dilutions in Tag dilution buffer:

The (5-7-95 Lig) — ~~later~~ previously determined to be 5 u/l, but it have lost activity over time



same dilutions also made w/ Tne from Blue Sepharose column pool

start runs w/ 20 uL of each dilution into 48 uL mix A ^{ref} p.102
10' 74°C, stop w/ 10 uL 0.5M EDTA, spot 20 uL
³²P x dCTP ref 7/14/95

SAM	CPM1
1 1/125	2730.00
2 1/250	1638.00
3 1/500	790.00
4 1/1000	388.00
5 1/2000	232.00
6 1/4000	143.00
7 1/125	9450.00
8 1/250	5021.00
9 1/500	2872.00
10 1/1000	1669.00
11 1/2000	899.00
12 1/4000	503.00
13 no enzy	67.00
C. Comb 7/22/95	

pmol 4 u/l
352 543 544 6.45 } av ~ 7.0 u/l
202 7.60
93 7.30

207 31.0 }
107 32 } 32.3 ave
56 33.8

(= 193600 units total in blue pool fr 13-17)

23.2
27.2 cpm/pmol
as of 7-22-95

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From Page No. _____

check conductivity after dialysis of P111

10 μ l / ml H₂Obuffer D 88 μ SHep col effluent 84 μ SDialysate 94 μ S

so conductivity is good and is similar to P183, 9 for TFA

1. Load on ~ 22 ml Hep equilibrated on with buffer D (P100)

at 0.67 ml/min (= 40 ml/hr = 2 col vol/hr)

2. wash 1 col vol3. Gradient (start ~ 9:30 AM)50 mM - 1.05 M KCl (0.67 ml/min)
using buffer D and E (2 M KCl)

so 0 - 50% E

600 ml total gradient vol (~ 30 col vol)

so 50 - 700 mM KCl is in 20 col vol same as P185, 9

0.5 ml/min span = 2A

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Storage buffer

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115

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2 L buffer F (as per 91342. PRP)

2 M Tris pH 8	40 ml	✓
0.5 M EDTA	0.4 ml	✓
0.1 M DTT	2 ml	✓
0.03 NP40 Pierce	✓	✓
0.1% Tween 20 Pierce	✓	✓
Glycerol	1 L	✓
H ₂ O		

2 L

Dialyze in 1 L for 5 hr. Change to another L for 5 hr.

Unit assay on fractions eluted from Heparin column:
do 1 dilution (1:150) of fractions # 35-53 and a series of
dilutions on the fraction with the maximum UV absorbance - #44

13 dilutions in Tag dilution buffer

Stack 298 μ l Tag dilution buffer p1000

1:150 for fractions 35-53, called H35-H53

and ~~1:125~~, 1:250, 1:500, 1:1000, 1:2000, 1:4000, 1:8000 of H44 - see dilutions on p 112

do make 1:125
but don't do
run w/ it

To Page No. _____

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From Page No. _____

pmol u/ml

fr 44 {
 1: 250 2180.00
 2: 500 938.00
 3: 1000 560.00
 4: 2000 398.00
 5: 4000 252.00
 6: 8000 146.00

274
114
55
44

10.5
8.5
5.5 } ave 9.53 u/ml

specific activity
= 19.1 cpm/p

Hop Fr
13150
dilution
35-53

no Enz
fr 35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53

21
22
23
24
25
26

57.00
1024.00
699.00
1128.00
1127.00
2104.00
2484.00
2621.00
4760.00
3657.00
3507.00
4717.00
4120.00
3517.00
3280.00
2282.00
1820.00
1301.00
750.00
513.00

pmole

412
748
574
550
740
447
552
515

9.27
16.82
12.92
12.39
16.7
14.6
12.4
11.4

$$\frac{412 \text{ pmole}}{2 \text{ ul}} \times 150 \times \frac{30}{10} = 9.27 \text{ u/ml}$$

$$\bar{x} = 13.3 \text{ u/ml} \pm 2.6 \text{ u/ml}$$

pool 41-48

(Pfrn) (1.34 ml/frn)
= 10.72 ml total

pooled frn 41-48
(~10.7 ml total vol)

Dealyze into buffer F
see P 115

Recovered 2.6 ml Tne after deanalysis (in by
add 2.6 ml buffer G of 4-29-55 (sup 6, 10 and 91342-1)

T Pag No

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J. Polansky

Date

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7-22-95

7-22-95 2A 0.67 ml/min
Hepatic Tm

7-22-95

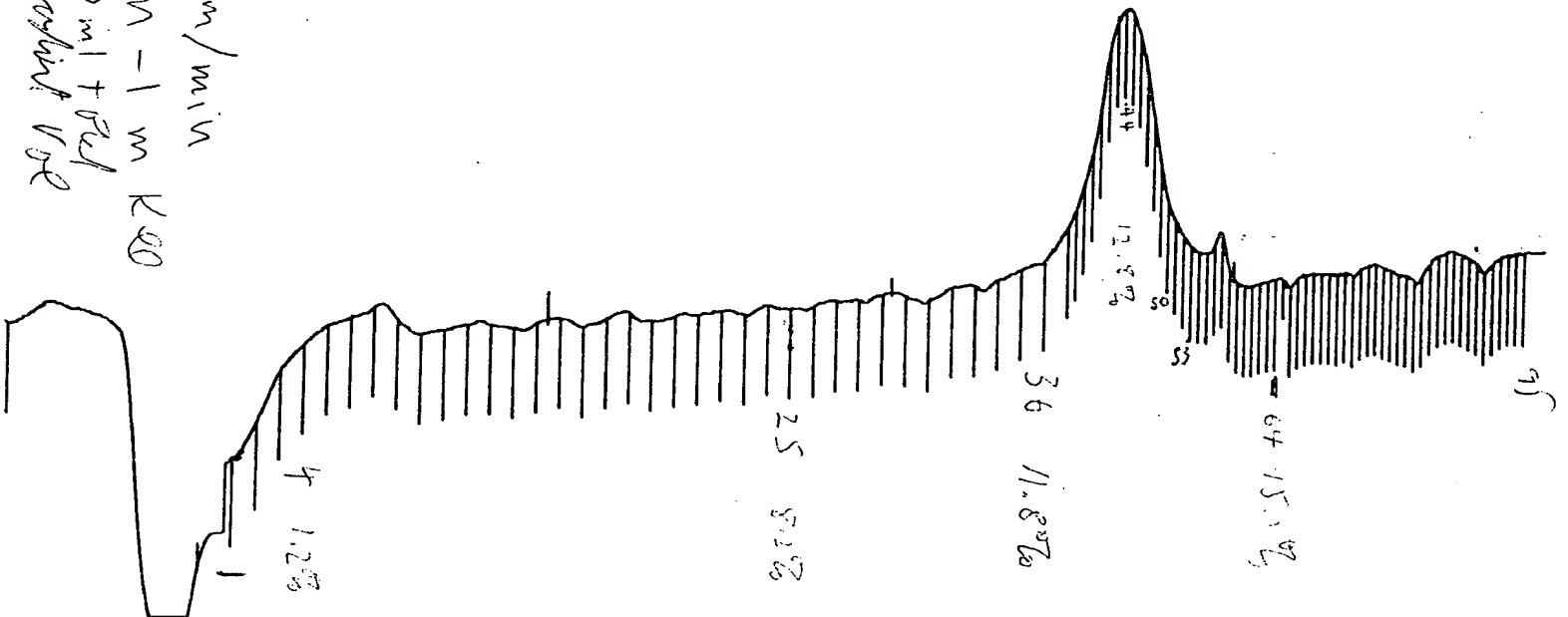
0.5 mm/min

50 mm - 1 m K20

600 ml + Red
Gravimetric

2 min
fix

→



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Project No. _____

Exhibit L-139

118

Book No. _____

TITLE _____

Appl. No. 09/558,421

From Page No. _____

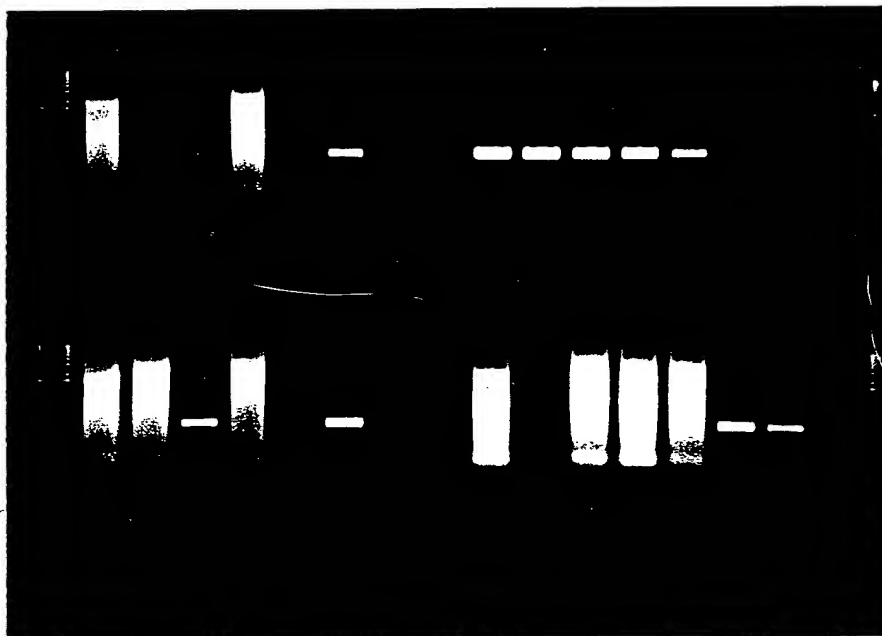
This experiment is detailed on p. 119-120, VB 11

The

Tag

J-7-95 L12

scale units	3	2.5	2.15	1	0.5	0.25	0.125



3 2.5 2.15 1 0.5 0.25 0.125

7-27-95

Blue
Seph

Heparin

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T Pag 1

7/24/95

R corded by

Barbara Damb

7-22-95

PCR rxns with Blue Sepharose and Heparin
fraction⁴ of Tne prep 7/22/95

age N — 7/23/95

ation of Blue Sepharose pool fractions and Heparin pool fraction of Tne.
Taq and Tne (5-7-95) prep will be tested in PCR alongside the new Tne.
an we use more than 2 units of new Tne and not get a smear?
ie 5-7-95 Tne prep gives a smear w/ more than 1 unit (p. 83 NB11)

tions:

Cheng buffer

15 cycles w/ old program ie lab 15 9600 #76 94°C 1min

est 0.125, 0.25, 0.5, 1, 1.5, 2, 2.5, 3 units

ake the 380bp product

50ul rxn

35x

94°C 30sec

55°C 30sec

72°C 2min

4°C

cocktail w/ all components except enzyme, for 34 rxns

340ul 5x Cheng

34ul 10mM dNTPs

34ul 50pg/ul m13 RF

34ul 20uM anchor primer > see p. 42 NB11

34ul 20uM 6681 primer

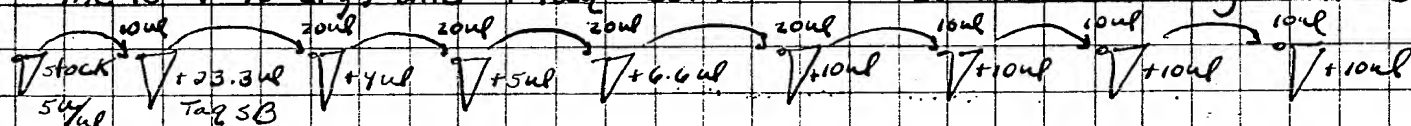
1156ul H₂O house distilled

1632ul

→ 48ul / PCR tube for 9600

enzyme dilutions in Taq SB:

or Tne (5-7-95 lig) and rTaq - both 50ul do the following dilutions

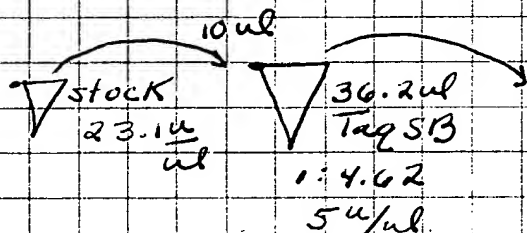


$D_f = 1.5^4 \text{ul}$ 1.25^4ul 1^4ul 0.75^4ul 0.5^4ul 0.25^4ul 0.125^4ul 0.0625^4ul

start rxns w/ 2ul of each dilution on ice, flick, spin down

for Blue sepharose pool - $23.1 \frac{\text{u}}{\text{ul}}$ when normalized to 5-7-95 Tne

ie. $\frac{5}{7} (32.3) = 23.1$ p. 112, NB11



same as Tne (5-7-95) dilutions

To Page No. _____

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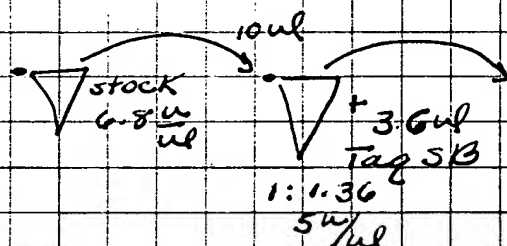
Carolyn Comb

Date

7/27/95

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Heparin fraction #44, which had peak UV absorbance
 is $6.8 \frac{\mu}{\mu l}$ when normalized to Tne (5-7-95 prep)
 i.e. $\frac{5}{7} (9.53) = 6.8 \frac{\mu}{\mu l}$ p. 116 NB 11



same dilutions as for Tne (5-7-95) p.

start rxns w/ $2 \mu l$ of each dilution starting w/ the
 $1.5 \frac{\mu}{\mu l}$ dilutions

35 cycles, stop w/ EDTA stop soln, $7.5 \mu l$
 run $25 \mu l$ on gel

Result on p. 118 NB 11 - new Tne prep (7-22-95)
 is not less prone to
 making a smear than the
 old (5-7-95) prep. So, DNA
 contamination of enz should
 not be Tne's main problem.
 Rather, Tne may have an
 intrinsic activity that makes
 it "smear" more easily than
 Tag

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Date

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Date

[Signature]

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Recorded by

[Signature]

7/27/95

T Page 1

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1) Can -Taq make a smear in high Mg^{2+} ? \pm primers \neq temp.

$\text{mix A} = \frac{49.4 \mu\text{l}}{510 \mu\text{l}} \text{H}_2\text{O}$

for 8 rxns

$\frac{80 \mu\text{l}}{16 \mu\text{l}} \times 10 \times \text{PCR buffer}$

$\frac{16 \mu\text{l}}{50 \text{pg}/\mu\text{l}} \times 50 \text{pg}/\mu\text{l} \text{ m13 RF target}$

$\frac{16 \mu\text{l}}{20 \mu\text{M}} \times 20 \mu\text{M} \text{ anchor}$

$\frac{16 \mu\text{l}}{4681} \times 4681 \text{ primer, } 20 \mu\text{M}$

$\frac{1.31 \mu\text{l}}{1.29 \mu\text{l}} \times 1.29 \mu\text{l} \text{ Taq}$

$\frac{5.28 \mu\text{l}}{8.28 \mu\text{l}} \times 8.28 \mu\text{l} \text{ H}_2\text{O}$

640 μl

mix B = 542.4 μ l H₂O
for 8 rxns 80 μ l 10x PCR bu
16 μ l 10mm dNTP
1.31 μ l
~~1.6 μ l~~ 1 Taq 5 μ l
29 μ l H₂O ✓
4 μ l
640 μ l ✓

start rxns by adding Mg^{2+}

#	1	2	3	4	5	6	7	8	9	10	11	12	13
H ₂ O	17.9	17	16	12	8	4	0	17.9	17	16	12	8	4

mix A ✓ 80 —————
= + primers
template

[illegible]

500mM MgCl₂ ✓ add last just before PCR

2.1	3	4	8	12	16	20	2.1	3	4	8	12	16
100μl rxns												

35 cycles

stop whole rxn w/ 11 uL stop soln w/ 10x CDTA p 79 ✓

T Pag N

Witnessed & Und rsto d by me,

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Invented by

Dat

Do/any

8/1/95

Recorded by

Recorded by *Paula Pomb*

7/27/95

g N _____

yes The work better (make more product) with a hot start?

mix C for 4.5 rxns = 90ul 5x Cheng ✓

301.5 ul H₂O

9ul 50pg/ul m13 RF in TC from 3/94

9ul 20uM anchor primer ✓

9ul 20uM 6681 primer ✓

9ul 10mM dNTPS ✓

427.5

7/22/95

real 19.0 u/w

1:30.5x dilution

6:183

177ul Tag SB

95ul
+ 5ul Tne 0.24ul

duplicate

hot

hot start

start

(15)

(16)

(17)

(18)

stop at 25, 30, 35 cycles

20ul
+ 3ul stop

added eny
after 11 94°C denaturation
→ H5X12

Can human spleen genomic DNA promote smear formation - bad seed?
no template, no primers

high Mg²⁺ - short smear
2.5 rxns condition

Low Mg²⁺ - long smear condition

25ul 10x PCR buffer ✓

187.5ul H₂O ✓

5ul 10mM dNTPS ✓

7.5ul 50mM MgCl₂ ✓

225ul

90ul D ✓

2.5ul H₂O ✓

2.5ul genomic DNA ✓

5ul Tne 0.2 u/w

(19)

90ul D ✓

5ul H₂O ✓

5ul Tne

(20)

25ul 10x PCR buffer ✓

189.75ul H₂O ✓

5ul 10mM dNTPS ✓

5.25ul 50mM MgCl₂ ✓

225ul

90ul E ✓

2.5ul H₂O ✓

2.5ul genomic DNA ✓

5ul Tne

(21)

90ul E ✓

5ul H₂O ✓

5ul Tne

(22)

remove 10ul at 15, 20, 25, 30, 35 cycles STOP tubes + 2ul stop

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To Page No. _____

From Page No. _____

4) Does Tne make a smear when 1 or 2 dNTPs are missing from rxn?

4 dNTP mix, 2.5 mM each \Rightarrow 20 μ l each 10 mM dNTP stock

3 dNTP mix, AGC 2.5 mM each \Rightarrow 20 μ l 10 mM A ✓
 20 μ l 10 mM G ✓
 20 μ l 10 mM C ✓
 20 μ l H₂O ✓

use
8 μ l of
mixes
each 100
PCR rxn
for C_F = 2

2 dNTP mix, GT 2.5 mM each \Rightarrow 20 μ l 10 mM G ✓
 20 μ l 10 mM T ✓
 40 μ l H₂O ✓

mix F = 200 μ l 10x PCR buffer ✓
 for 20 rxns
 1480 μ l H₂O ✓
 1680 μ l

for 9.5 rxns

[G] 798 μ l ✓
 28.5 μ l 50 mM MgCl₂ ✓
 883.5 μ l

[H] 798 μ l ✓
 19.95 μ l 50 mM M ✓
 8.55 μ l H₂O ✓
 883.5 μ l

20 μ l G 20 μ l G 20 μ l K
 24 μ l 4 dNTP 24 μ l 3 dNTP mix
 = 3 dNTP mix

695 μ l
 + 5 μ l Tne . 24 μ l

23, 24 25, 26 27, 28

100 μ l 35 cycles
 11 μ l stop (p. 79)

Lab 16, 9600 method 103 1:25 P^m

29, 30

31, 32

33, 3

With ss d & Und rst d by me,

Bobolung

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8/1/95

Inv nt d by

R cord d by

Dan Romb

Dat

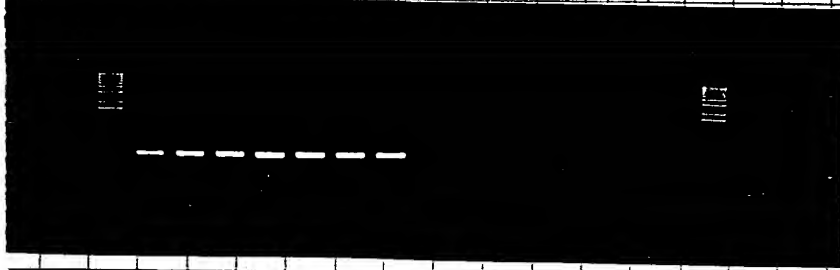
7/27/95

T Page N

ag N _____

2% agarose gel \Rightarrow 3.6g agarose
 300mL 1X TAE
 20uL CtBr 598g

target + primers no input DNA
 Taq
 NgCl₂ 1.05 1.5 2 4 6 8 10 1.05 1.5 2 4 6 8 10

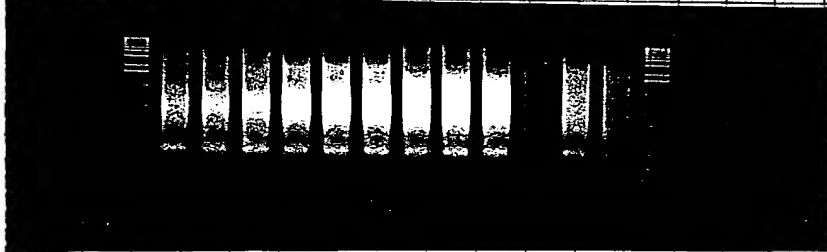


C. Comb
 7/25/95

Results

- Increasing Mg^{2+} did not cause Taq to make a smear either in the presence or absence of input DNA

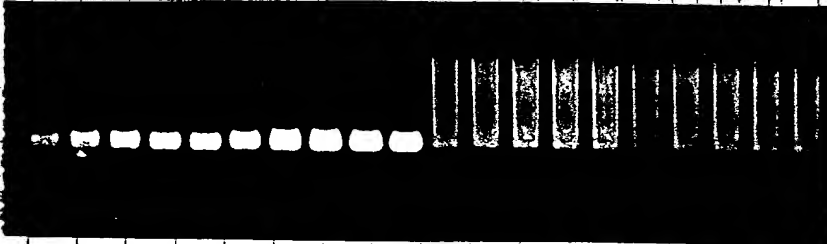
cycles
 25 30 35 25 30 35 25 30 35 25 30 35
 Tne (7-22-95 prep) \rightarrow Cheng buffer
 hot start cold start primers + target
 3uL/bowl rxn



C. Comb
 7/28/95

- With 3 units Tne, the hot start rxns did not smear any less than cold start rxn. I should have used 1.36 units in order to get product instead of smear redo this expt w/ 1.36 units Tne rxn

1.5 mM MgCl₂ Tne (7-22-95) 1.05 mM MgCl₂
 genomic genomic genomic genomic
 15 20 25 30 35 15 20 25 30 35 15 20 25 30 35 15 20 25 30 35



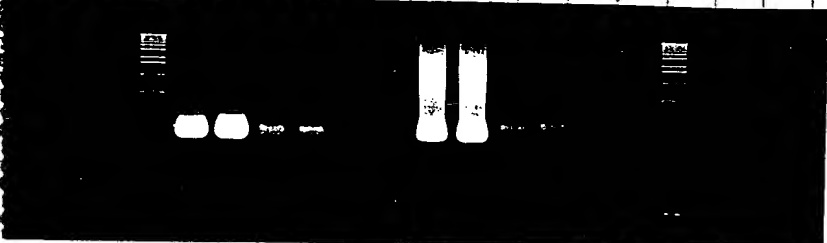
PCR buffer
 3uL/rxn \rightarrow too much

C. Comb
 7/28/95

- Addition of genomic DNA did not result in less smear over time w/ 3 units Tne

redo this expt w/ 1.36 units Tne rxn

present
 1.5 mM MgCl₂ Tne (7-22-95) 1.05 mM MgCl₂ \rightarrow PCR buffer
 4 4 3 3 2 2 4 4 3 3 2 2 35 cycles 3uL/rxn



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- 3 dNTP mix = AGC present
- 2 dNTP mix = GT present

- No smear made when 2 dNTPs (C+A) are missing, so smear is probably not made by a TdT activity.

To Page No. _____

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Polamp

Date

8/1/95

Invented by

Recorded by

Carolyn Comb

Date

7/27/95

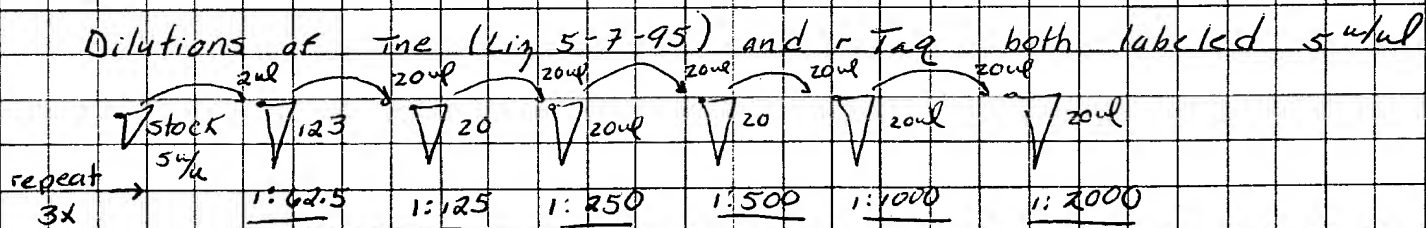
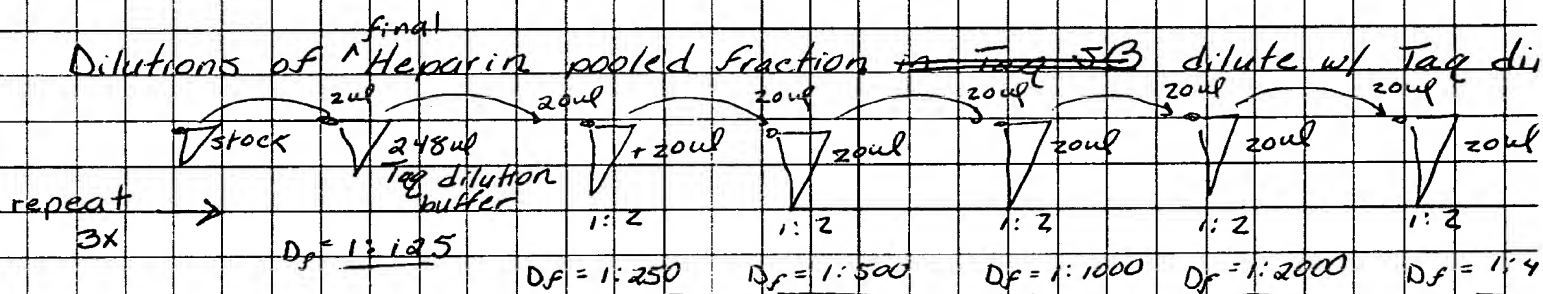
From Page No. 7/26/95 Unit assay of new Tne prep (7-22-95) after dialysis into Tag storage buffer.

- old 5-7-95 Tne prep & r Tag will be done too
- 3 replicates of each dilution series, done 3x independ

cocktail: 200 μ l of Taps, $MgCl_2$, KCl mix -
 2,520 μ l H_2O w/ p1000
 60 μ l 10mM dNTPs
 405 μ l 3.7 mg/ml gapped DNA
 9.3 μ l ^{32}P dCTP ref date 7/14/95
3.2 mL

use 48 μ l per rxn

start rxns by adding 2 μ l of enzyme dilutions on ice.



- rxns stopped w/ 10 μ l 0.5M EDTA
- 20 μ l of each rxn spotted on GFC filters

SAM CPM1 ave ave
pmol u/ul
C. Combs
7/27/95

Project No. _____
B ok No. _____

The 7-22-95

1	8081.00		
2	7346.00	625	11.7
3	5871.00		
4	5139.00		
5	5341.00	441	16.5
6	4559.00		
7	3009.00		
8	2963.00	255	19.1
9	2724.00		
10	1492.00		
11	1359.00	125	18.8
12	1429.00		
13	891.00		
14	899.00	79	
15	895.00		
16	490.00		
17	402.00		
18	524.00		

The
7-22-95
 $\frac{19.0 \text{ u}}{\text{ave}}$

$\Rightarrow \text{use } (x \text{ ul})(19.0) = 1.36 \text{ u/100ul}$
 $\Rightarrow 0.0716 \text{ ul/100ul PCR rxn}$

The 7-22-95

19	6160.00		
20	6476.00	582	5.4
21	7195.00		
22	4215.00		
23	4266.00	354	6.6
24	3596.00		
7	2124.00		
8	2014.00	181	6.8
9	2055.00		
10	1160.00		
11	998.00	95	7.0
12	1024.00		
13	572.00		
14	610.00		
15	609.00		
16	361.00		
17	352.00		
18	348.00		

The
Liz
 $\frac{6.8 \text{ u}}{\text{ave}}$

\Rightarrow we had been using 0.2 ul/100ul rxn
0.2 ul is really 1.36 units, not unit
as we thought based on the
unit value 5 u/ul

The 7-22-95

19	8453.00		
20	6925.00	658	6.17
21	7075.00		
22	4769.00		
23	3803.00	387	7.25
24	4613.00		
25	2896.00		
26	2565.00	240	9.00
27	2722.00		
28	1185.00		
29	1404.00	115	8.60
30	1334.00		
31	1234.00		
32	873.00	89.7	13.40
33	953.00		
34	592.00		
35	527.00		
36	509.00		

Tag
 $\frac{8.28 \text{ u}}{\text{ave}}$
 \uparrow
expected
5 u/ul on
real units
in nmol/30'

$\Rightarrow \text{use } (x \text{ ul})(8.28 \text{ u/ul}) = 1.36 \text{ u/100ul}$
 $= 0.164 \text{ ul/100ul PCR}$

The 7-22-95

37	56483.00		
38	57656.00	56855	34.1 CPM
39	56427.00	ave	pmol

To Page No. _____

& Understood by me, Polarp	Date 8/1/95	Invent d by 	Date 7/27/95
	Recorded by Carolyn Combs		

Proj ct No. _____

Book No. _____

TITLE

JU containing templates
(Myron Goodman assay)

From Page No. _____

Exhibit L-141

Appl. No. 09/558,421

7-11-95
"Fidel pri" (27mer)

CGAGACATGGCGTCCAGTCACGACCT
GCTCTGTALCGCAGGGTCAGTGCTGGACTAGTACGAGCTACT

27 bp


"Fidel-Templ"
or "Fidel-Templ"
(7-11-95)
(42mer)


#F1351

This is not old "Fidel T"
of PJ110. This new one
less stringent for primer
according to oligo program

1. ssDNA region is same as MB JBC Crigleton & MG R
for + dGTP + dATP (get G-A mismatch at position 3 and)
+ dCTP for reverse

2. For test of dATP incorp opposite Template JU,
have all 4 dNTPs present at 200 μ M each
and look for pause one site before JU
(run on 7th PAGE?)

G
C
A ↑
U T A
G C (31) 
A T
T A
C G

primer (27) 

To Page No

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Dat

Invented by

Date

Deanna Dole

8/1/95

R c rded by

7-28-95

JU vs JT in template

From Page N _____

① ② ③ ④ ⑤ ⑥ ⑦ ⑧

³²P pri. ofide / Temp JT
50 mM primer (PST)

4 →

10 mM

³²P pri. ofide / Temp JU
250 mM primer (PST)

4 →

~ 10 mM
total10X Tag PCR buffer
50 mM MgCl₂10 10
3 310 10
3 31.5 mM
Cl⁻

10X Vent buffer

10 10

10 10

10 mM dNTPs

2 μl →

2.5 mM
dNTP

rTag 0.0625 μM

2

2

Tne 0.0625 μM

2

2

0.125 μM

Vent 0.0625 μM

2

2

0.013 μM
pol m

DeepVent 0.0625

2

2

so ~
primer
polH₂O

79 →

72 →

79 →

72 →

so has
primer
overpopreheat to 70°C
Start with addition of pol.

remove 10 μl to 5 μl cycle seq stop at

0 5 10 20 40 60 90

To Page No _____

Witnessed & Understood by me,

D. D. Camp

Date

8/1/95

Initiated by

R. C. R. d. by

Date

7-27-95

age N _____

sequence Rxn same as P 27, 4 and 90 11
 using ³²P pri fidel Temp with ST und du

To Page No. _____

s d & Understood by me,

Polkay

Date

8/1/95

Invented by

Recorded by

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7-27-95

Project No. _____

Book No. _____

TITLE Effect of annealing temperature on the smear

128

From Page No. _____

- anneal at 50°C, 60°C, 70°C w/ 72°C extension - no input DNA
- take out rxn aliquots after 10, 15, 20, 25, 30, 35 cycles
- use 1.36 units Tne (5-7-95 Lig) / 100ul rxn
- test the effect of annealing temp on both the long (low Mg²⁺) and short (hi Mg²⁺) smears

mix A = high Mg²⁺ for small smear - enough for 3.5 rxns

280ul H₂O ✓
 35ul 10x PCR buffer ✓
 7ul 10mM dNTPs C_f = 200uM ✓
10.5ul 50mM MgCl₂ C_f = 1.5mM ✓
 332.5ul

mix B = low Mg²⁺ for long smear - enough for 3.5 rxn

283.15ul H₂O
 35ul 10x PCR buffer ✓
 7ul 10mM dNTPs
7.35ul 50mM MgCl₂ C_f = 1.05mM
 332.5ul

* 25 fold dilution
 The stock (Lig: 5uM (real units p. 127)
 3ul Tne
 72ul Tag
 75ul of 0.

annealing temp	50°C	60°C	72°C	70°C	
	Lab 15	Lab 16	Lab 562		
rxn #	1	2	3	4	5
1 ul Mix A hi Mg	95		95		95
1 ul Mix B low Mg		95		95	95
* ul Tne 0.24ul 5-7-95 Lig	5				
	100ul rxns				

596 received mix that were made up a different time to the mix A+B for 1

remove 10ul of rxn to 2ul STOP soln w/ EDTA p. 79 NB11
 at cycles 10, 15, 20, 25, 30, 35

T Page 1

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OS Olamp

8/1/95

R corded by

Daniel Poms

7/28/95

ag N. _____

cling Lab 15 program 76 94° 1min
 35x { 94° 30sec
 50° 30sec
 72° 2min
 4° —

Lab 16 program 103 94° 1min
 4.5 min per cycle 35x { 94° 30sec
 60° 30sec
 72° 2min
 4° —

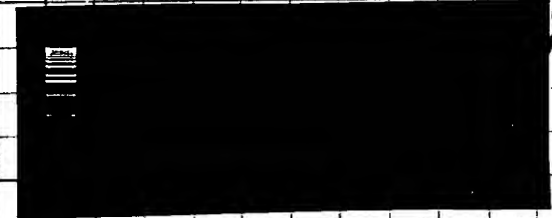
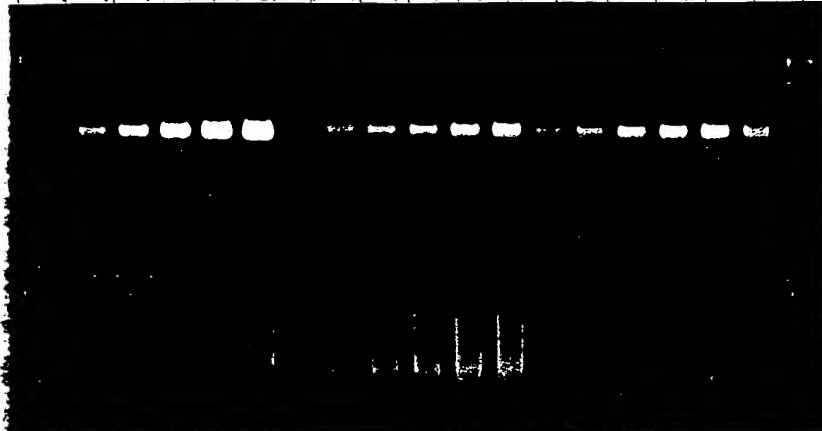
S&I program 133 94° 1min
 4.5 min per cycle 35x { 94° 30sec
 72° 2min 30sec
 4° —

2 temp. PCR 35x { 94° 30sec
 72° 2min 30sec
 4° —

2% agarose gel, 36 samples + primers
 2ul 20mM Tris, 2ul 20mM NaCl, 94ul H₂O, 1ul stop
 12 short 50° 1-35, 3-10 to 3-35, 5-10 to 5-35, 7-10 to 7-35
 12 long 50° 2-10 to 2-35, 4-10 to 4-35, 6-10 to 6-35
 primers, 7/15/95 Tne, 7/22/95 Tne
 1.5ul Tne Lig, 3ul 50% gly, 6.5ul H₂O, 11ul stop, 10ul 20mM Tris

Tne (5-7-95 Lig) 1.36 uL/min

ing 50°C 60°C 72°C
 10 15 20 25 30 35 10 15 20 25 30 35 10 15 20 25 30 35



Comb
 12845

To Page No. _____

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Date

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7/28/95

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8/1/95

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 Comb

Project No. _____

Book No. _____

unit array for 1.1X Tag
TITLE same as P121, 9 and P52, 10

From Page No. _____

From Page No.	Rxn#	ul/assay	1% Tween 20/NP40
4°C #10 (P121, 9) (no det)	1-3	2	1 ul
11 1.1X	4-6	3.64	
1 (2X 0.1% TN)	7-9	2	
14 (2X TFI buffer)	10-12	2	
Tag (same as P121, 9) K5 dil	13-17	2	
From P121 1.1X called "new" on P34 (58.95)	18-20	2	
Room Temp #11 1.1X	21-23	2.64	
-20 5 months on P52 (see Rxn# 21-23 on P52) got 5-4% recovery (on P53)	24-26	2	

Exhibit L-143

Appl. No. 09/558,421

°C	SAM	CPM1	ave	ul/ul	% of zero time P122, 9
10	1	1779.00			
	2	1970.00	2029	.008	38%
	3	2337.00			
11	4	8375.00		.033	103
	5	8284.00			
	6	8267.00			
1	7	10246.00	9774	.039	105
	8	9851.00			
	9	9556.00			
14	10	8959.00			
	11	9908.00	9484	.037	107
	12	9584.00			
Tag	13	10530.00			
	14	9527.00	10,119	(.04 by definition)	
	15	9706.00			
	16	9859.00			
	17	10773.00			
20	18	6924.00	7063	.028	(was .025 on P53 so >100%)
25	19	7046.00			
	20	7219.00			
31	21	6156.00			
	22	6520.00	6257	.025	77% (P154, 9 is 0 time po (P38, 10))
	23	6095.00			
30°C	24	5038.00			
25	25	4980.00		.019	$\frac{.017}{.030} = 63\%$ recovered see P53
25	26	4755.00			
30°C	27	82.00			
25	28	119284.00			
25	29	121726.00			

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Inv nt d by

Date

To Pag

D. Poling

8/11/95

R c rd d by

7-31-95

Project No. _____

Book No. _____

TITLE Hot vs Cold start PCR w/ Tne (5-

130

From Page No. — Is more ^{specific} product made and less smear in a hot vs cold start
 Look at products at 10, 15, 20, 25, 30, 35 cycles

• start 100ul rxns w/ 2ul enz in order to keep [glycerol] low \Rightarrow SB + 50%, rxns will be 1%, do duplicate hot + cold start rxns

• materials: ~~5x stock of Tne~~

[A] mix: 100mM Tricine pH 9 \Rightarrow 100ul 1M Tricine pH 9 ✓
 5.25mM MgOAc 5.25ul 1M MgOAc ✓
 4.25mM KOAc 212.5ul 2M KOAc ✓
 682.25ul H₂O ✓
 1ml

cocktail for 4.5 reactions = 90ul 5x [A] mix ✓
 315ul H₂O ✓

[B]
 9ul 50pg/ul M13RF ✓
 9ul 20uM anchor primer ✓
 9ul 20uM 4681 primer ✓
 9ul 10mM dNTPs ✓

441ul

98ul B
 2ul Tne 0.68uM

98ul B
 2ul Tne

98ul B
 2ul Tne

98ul B
 2ul Tne

①

②

③

④

cold start

hot start - em a
 2:40PM denati
 of 1st

Tne dilution: 3ul Tne (5-7-95 Lig) 0.8uM ✓
 27ul Tne SB ✓

30ul of 0.68uM Tne

T Pag N

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Inv nt d by

Dat

R cord d by

7/31/95

Bobolamp

8/1/95

Paulen Pomb

ag N _____

move 10ul after 10, 15, 20, 25, 30, 35 cycles + add 2ul STOP w/ 100mM EDTA
 ab 14 9600 program 10.3 = 94°C 1min

35x { 94°C 30sec
 55°C 30sec
 72°C 2min
 4°C —

2% agarose gel

top

→ 1-10C 10 1-35C 4-35C } 2
 25 19-10C 22-35C } 2

lit:

Tne (5-7-95 Liz prep) 1.36 w/rxn

Cold Start - duplicates

Hot Start - duplicates

cycle#

10 15 20 25 30 35 10 15 20 25 30 35 10 15 20 25 30 35 10 15 20 25 30 35

buffer = 20mM Tricine

85mM KOAc

1.05mM MgCl₂3116
2C

- 380bp m.3 product,

conclusion: Hot start did not result in more specific product.
 One cold start duplicate rxn failed, no apparent reason.

To Page No. _____

ss d & Und rstood by me,

Date

Invented by

Dat

Polansky

8/1/95

Recorded by

Paula Cumb

7/31/95

From Page No. 123

same as p. 123 & 3 except only 1.36 μ l of Tne (5-7-95) will be used per rxn

* 19 90 μ l D ✓
5.5 μ l H₂O ✓
2.5 μ l genomic DNA ^{5/6/95} H5X2 ✓
2 μ l Tne 0.68 μ l p. 130

* 20 90 μ l D ✓
8 μ l H₂O ✓
2 μ l Tne

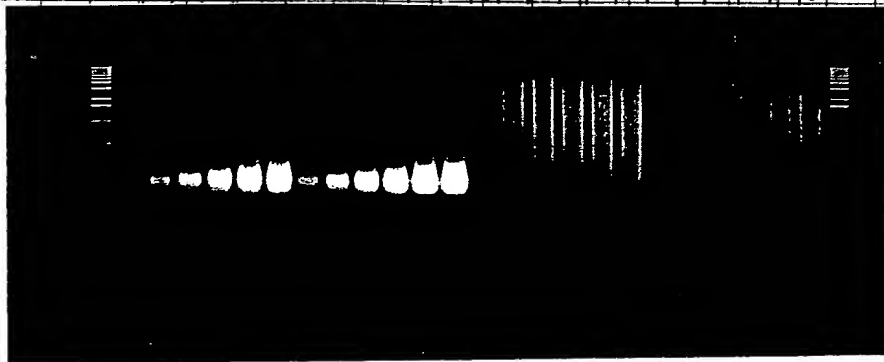
* 21 90 μ l E
5.5 μ l H₂O
2.5 μ l g
2 μ l Tne

* 22 90 μ l E ✓
8 μ l H₂O ✓
2 μ l Tne

10 μ l aliquots removed after 10, 15, 20, 25, 30, 35 cycles + 2 μ l stop w/ 100 mM Tne 1.36 μ l/rxn (5-7-95 Lig prep) - no pc prese.

Result:

1.5 mM MgCl₂ + genomic - genomic
1.05 mM MgCl₂ + genomic - genomic
cycle # → 10 15 20 25 30 35 10 15 20 25 30 35 10 15 20 25 30 35 10 15 20 25 30 35

7/31/95^{cc}

Conclusion: 1) The smear is visible even after 10 cycles - we should run aliquots of earlier cycles to determine when the smear becomes visible.

2) The 1.05 mM Mg²⁺ long smears are more intense with genomic DNA present than without genomic DNA. T may be a real result or it may be variation in rxns - repeat w/ triplicates

bit of support for idea that genomic DNA can act as a bad seed.

With ssed & Und rst d by m ,

Date

Inv nt d by

Dat

8/1/95

R cord d by

7/31/95

Polansky

Paulen Pumb

Page No.

$[Mg^{2+}]$ titration in a short PCR rxn w/ Tne

(5/7/95 Liz)

Page No. _____

purpose: To demonstrate that lowering $[Mg^{2+}]$ shifts the size of DNA products in smear from small to longer \rightarrow ~~in~~ in a short PCR rxn w/ Tne alone. This result has been observed w/ ~~the~~ 1.5mM Mg^{2+} vs 1.05mM Mg^{2+} , but the range of concentrations has not been tested. Does the transition occur over a narrow or broad range of Mg^{2+} ? What happens w/ 85mM KOAc + 1.5mM Mg^{2+} ?
 ↳ we have not tested this condition before

real expt conditions:

Cf $MgOAc$ (mM) 0.9, 1.05, 1.1, 1.15, 1.2, 1.3, 1.4, 1.5, 1.7 (9 levels)

Cf KOAc (mM) 50, 85 (2 levels)

template and primers for m13 380bp product present

35 cycles, 55°C annealing temp

1.36 μ /rxn rxn Tne - 5-7-95 Liz prep

start rxns w/ Mg^{2+}

materials: 25mM $MgOAc \rightarrow \frac{25mM \cdot 1000\mu L}{1000mM} = 25\mu L$ 1M $MgOAc$
 975 μ L H_2O

A - 50mM KOAc for 9.5 rxns

mix B - 85mM KOAc 9.5 rxns

19 μ L 1M Tricine pH 9.0 ✓

23.75 μ L 2M KOAc ✓

734.35 μ L H_2O ✓

19 μ L 10mM dNTPs Cf=200 μ M ✓

19 μ L 20 μ M 6681 primer ✓

19 μ L 20 μ M anchor primer ✓

19 μ L 50% m13 RF ✓

1.9 μ L Tne (5-7-95 Liz prep) \approx 1.36 μ /rxn

855 μ L \leftarrow 6.8 μ /L

19 μ L 1M Tricine pH 9 ✓

40.375 μ L 2M KOAc ✓

717.725 μ L H_2O ✓

19 μ L 10mM dNTP ✓

19 μ L 20 μ M 6681 ✓

19 μ L 20 μ M anchor ✓

19 μ L 50% m13 ✓

1.9 μ L Tne 5-7-95

855

top of gel

1 2 3 4 5 6 7 8 9 10-18

no tube 9 - I dropped it

there is a tube 18

12 PM - 1:30 PM

Lab 16 9600 10.3

90 μ L

90 μ L

6.4 5.8 5.6 5.4 5.2 4.8 4.4 4 3.2 same series as 1-9

2 $MgOAc$ 3.6 4.2 4.4 4.6 4.8 5.2 5.6 6 6.8

add Mg , mix well & keep on ice til cycling

100 μ L rxns

stop w/ 100mM EDTA, run 200 μ L on 1.2% agarose gel

To Page No. _____

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Date

Veronica Polay

8/7/95

Recorded by

8/1/95

Paula

Addition of genomic DNA to a Tne short PCR rxn - 3, 6, 10, 12, 15, 20 cycle aliquot

ag N.132

purpose: To determine if adding human genomic DNA to a Tne PCR rxn leads to production of a smear by an earlier cycle # than without genomic DNA. Genomic DNA ^{200ng/100ul rxn} might act like more "bad seed" material and exacerbate the smearing rxn. This experiment was tried on p. 132. Cycle #'s 10-35 were run on the gel. There was an indication that addition of genomic DNA made the smear darker by an earlier cycle #. Now, we are repeating the p.132 exp in triplicate and looking at even earlier cycle #'s.

summary of experimental cond: 3 rxns w/ human spleen DNA, 3 without
materials:

no primers added

start rxns by adding 1.36ul/100ul rxn w/
Tne (5-7-95 Lig prep)55°C annealing temp program 103, Lab 10
200ng of human spleen DNA/rxn

materials: mix A: for 7.5 reactions = 75ul 10x PCR buffer
56.9.25ul H₂O
15ul 10mM dNTPs
15.75ul 50mM MgCl₂
Tne (5-7-95 dilution
2ul Tne
15ul 5.0
20ul 0.68
Tne

	top of gel				bottom of gel		
	1	2	3		4	5	6
A	90ul	—	—		—	—	—
1	5.5ul	—	—		8ul	—	—
2	2.5ul	—	—		none	—	—
3	2.5ul	—	—		—	—	—
4	2.5ul	—	—		—	—	—
5	2.5ul	—	—		—	—	—
6	2.5ul	—	—		—	—	—
7	2.5ul	—	—		—	—	—
8	2.5ul	—	—		—	—	—
9	2.5ul	—	—		—	—	—
10	2.5ul	—	—		—	—	—
11	2.5ul	—	—		—	—	—
12	2.5ul	—	—		—	—	—
13	2.5ul	—	—		—	—	—
14	2.5ul	—	—		—	—	—
15	2.5ul	—	—		—	—	—
16	2.5ul	—	—		—	—	—
17	2.5ul	—	—		—	—	—
18	2.5ul	—	—		—	—	—
19	2.5ul	—	—		—	—	—
20	2.5ul	—	—		—	—	—
21	2.5ul	—	—		—	—	—
22	2.5ul	—	—		—	—	—
23	2.5ul	—	—		—	—	—
24	2.5ul	—	—		—	—	—
25	2.5ul	—	—		—	—	—
26	2.5ul	—	—		—	—	—
27	2.5ul	—	—		—	—	—
28	2.5ul	—	—		—	—	—
29	2.5ul	—	—		—	—	—
30	2.5ul	—	—		—	—	—
31	2.5ul	—	—		—	—	—
32	2.5ul	—	—		—	—	—
33	2.5ul	—	—		—	—	—
34	2.5ul	—	—		—	—	—
35	2.5ul	—	—		—	—	—
36	2.5ul	—	—		—	—	—
37	2.5ul	—	—		—	—	—
38	2.5ul	—	—		—	—	—
39	2.5ul	—	—		—	—	—
40	2.5ul	—	—		—	—	—
41	2.5ul	—	—		—	—	—
42	2.5ul	—	—		—	—	—
43	2.5ul	—	—		—	—	—
44	2.5ul	—	—		—	—	—
45	2.5ul	—	—		—	—	—
46	2.5ul	—	—		—	—	—
47	2.5ul	—	—		—	—	—
48	2.5ul	—	—		—	—	—
49	2.5ul	—	—		—	—	—
50	2.5ul	—	—		—	—	—

move 10ul to a tube on ice w/ 2ul STOP soln in it (100mM EDTA)
3, 6, 10, 12, 15, 20 cycles, run 10ul on 1.2% agarose gel

6 + 6-9 = 3ul 6-9 went into 6-6C stop tube c = cycle #

See result on p. 134

To Page No. 134

s d & Und rsto d by me,

Date

Invented by

Date

created a Polypep

8/1/95

Record d by

8/1/95

From Page No. _____

Tne (5-7-95 Lig prep) 1.36 μ /rxn, 100ul rxn w/ template + primers

MgOAc (mM)

0.9 | 1.0 | 1.1 | 1.5 | 1.2 | 1.3 | 1.4 | 1.5

50mM
KOAc85mM
KOAc

• 1.3mM MgOAc was optimal for making product - note that we have made pre w/ 1.05mM Mg^{2+} in earlier e when glycerol + ~~1.05mM~~ also pre.

• 50mM KOAc is not sufficient for product formation, but 85mM KOAc is - values between 50 + 85 have not been tested

• The size of DNA products in sme does vary from small to longer as $[Mg^{2+}]$ varies from 1.5 - 0.9mM Mg

triplicate rxns w/ Tne (5-7-95 Lig prep)

(see P135 for reaction)

replicate 1 replicate 2 replicate 3

cycle # 3 | 6 | 10 | 12 | 15 | 20 | 3 | 6 | 10 | 12 | 15 | 20 | 3 | 6 | 10 | 12 | 15 | 20

Conclusion.

+ 200ng human
spleen genomic
DNA per 100ul rxn

There maybe a 1x
amount of contam.
DNA still (or RNA
in TNE prep -
3-4 cycles needed
see smear

no genomic DNA

e. emb
3/2/95

T Page 1

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Date

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Date

Dennis Polay

8/7/95

Recorded by

Dennis Polay

8-2-95

From Page No. _____

purpose: To determine if Mg²⁺ controls Long PCR smear size and to see if the smear is primer/template independent. Note that Long PCR rxn uses different buffering components than we've been using for st.

In a single Tne enzy PCR of 330 bp product, 1.2 mM Mg²⁺ - 1.3 mM is at the center of transition from small to long smear is optimal for product formation if 85 mM KOAc present.

do: 7 levels of Mg²⁺, ± target and primers, 2 ratios of Tag: Tne

materials: LTI's Tag Long PCR system + Kalas recommendations for 7-22-95 Tne prep

dilution of Tne in Tag SIB: 2 ul Tne (7-22-95 prep, 19^u/ul)

① 59.64 ul Tag SIB ✓ 1:30.82
mix

② 2 ul of dilution ①
59.64 ul Tag SIB 1:30.82

61.64 ul of 0.02^u/ul Tne

enzyme mixes:

Final Tag (u)	Tne (mM)	Tag (5 ^u /ul)	Tne (0.02 ^u /ul)	SIB
1	1	4 10	1 2.5	15 3
1	10	4 10	10 25	8 1
1	2	2 5	4 2.5	7 1

mix 1 with primer and template for 16 rxns: 14 ul 10 mM dNTP

14 ul primer mix 1

14 ul genomic temp

27.2 ul H₂O

320 ul

mix 1 without primer + template, 16 rxns:

14 ul 10 mM dNTP

304 ul H₂O

320 ul

T Page N

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Date

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Record d by

8/12/95

Dancer Backup

Pauler Puh

ag No. _____	Mg ²⁺ mM				
2 (1:1 TT) 1 st for 5 rxns =	50	✓	50 ul 5x A	25 ul	
122: Tne			0 & 30 ul 5x B	1	
			5, 5 ul 1u Tag: 1mU Tne	(7-22-95 prep)	
			95 (95 ul H ₂ O)		
			150		
2 (1:1 TT) 1 st =	45	✓	45 ul 5x A		
mM Mg ²⁺	5	✓	5 ul 5x B		
			5 ul 1u Tag: 1mU Tne	(200 ul H ₂ O eng mix)	
			95 ul H ₂ O		
			add H ₂ O		
2 (1:1 TT) 10.2 mM Mg ²⁺ =	40	✓	40 ul 5x A		
			10 ul 5x B		
			5 ul 1u Tag: 1mU Tne		
			95 ul H ₂ O		
2 (1:1 TT) 1.3 mM Mg ²⁺ =	35	✓	35 ul 5x A		
			15 ul 5x B		
			5 ul 1u Tag: 1mU Tne		
			95 ul H ₂ O		
2 (1:1 TT) 1.4 mM Mg ²⁺ =	30	✓	30 ul 5x A		
			20 ul 5x B		
			5 ul 1u Tag: 1mU Tne		
			95 ul H ₂ O		
2 (1:1 TT) 1.5 mM Mg ²⁺ =	25	✓	25 ul 5x A		
			25 ul 5x B		
			"		
2 (1:1 TT) 1.6 mM Mg ²⁺ =	20	✓	20 ul 5x A		
			30 ul 5x B		
			"		
2 (1:1 TT) 1.6 mM Mg ²⁺ =	20	✓	20 ul 5x A		
			30 ul 5x B		
			"		

Take each of these mixes again with the 1:10 TT mix
 and a mix 2 (1:2 TT) 1.6 mM Mg²⁺

To Pag No. _____

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Dat

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Date

Barbara Polansky

8/7/95

R cord d by

8/2/95

Barbara Polansky

From Page No.____

[illegible]

15 94°C 30 sec
 35 cycles { 94°C 30 sec
 { 60°C 30 sec
 { 68°C 10 min
 4°C

Step 1 Full stepwise containing 100mM EDTA (p.79)
200mM in 0.7% agarose gel, 100V 6'30" - 7^{pm}

With ss d & Und rstood by me,

Donal a Polay

Dat .

8/7/95

Inv nted by

Record of by

Recorded by *Paula [unclear]*

Date

8/2/95

To Page **M**

Project No. _____

Book No. _____

TITLE

32P end label Pri 2 - ... Pri 2
for test of decreasing primer length

From Page No. _____

mix for 14 labeling rxns: 28 μ l 32 P ATP (3.33 μ M) ref. 8/4/95 (6.66 μ l per rxn)
 28 μ l 5x Kinase buffer
 4.2 μ l PAK - new lot
 56.56 μ l H_2O
 140 μ l

rxns = 1. 66 μ l of each oligo - Fidel pri 2, 3, 4, 6, 8, 10, 12, 14, 16, 18
 8.34 μ l mix \leftarrow 10 μ M stocks (16.6 pmol primer total)
 10 μ l in 9600 tubes
 37 $^{\circ}$, 30' in Lab 14 9600
 55 $^{\circ}$, 5' "
 cool to 4 $^{\circ}$ C before opening tubes

add 2 μ l 10 μ M Fidel temp dT - 20 pmol total
 80 $^{\circ}$ C, 5'
 cool to Room temp, 15 min 12 9600

add 64.7 μ l 10 μ M Tris pH 8.0
 stored at -20 $^{\circ}$ C overnight

use 42/1000 reaction for 10 μ M primer

template = 20 pmol = 1.2
 pri 16.6 pmole

To Page N

Witness d & Und rst d by m ,

D. S. Polansky

Dat

8/7/95

Inv nt d by

R cord d by

Paula L. Smith

Dat

8-2-95

TNE

3g No. _____

lig

Q14 / SmaI / SphI .003 pmol/.1
 b. H3 / Gilled in / SphI .015 pmol/.1
 x ligation buffer
 H₂O
 Ligate (10)

2
 1.5
 1
 4
 12.5
 1
 20.1

RT - 30 min.

Jason returned 2.1 of the lig with 100.1 DH10B cc.
 std xform. Plated 10% + 90% on yet amp plates. 37°C ON

#2 10% 90%
 18 ~150

picked 8 colonies into 3 mls of CG + ampic 37°C - ON

is mp as usual. Dissolved in 50 µl TE.

mp 3
 DRRG 2
 H₂O 13
 BpHTE 1
 E. coli 1
 10

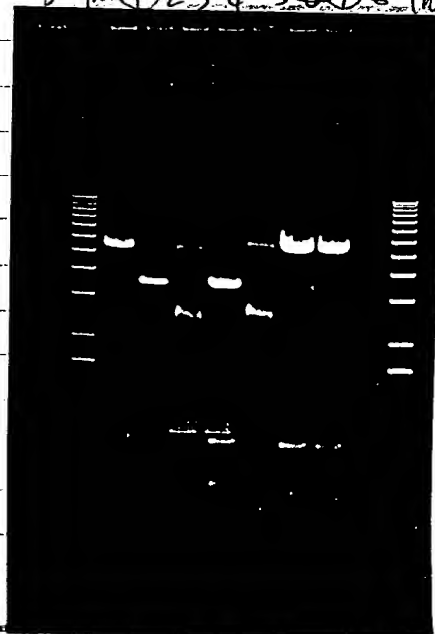
37°C - 1 hr.

Applied to a
 0.8% agarose
 gel. Gel
 run at 100V

4575
 2000
 6575

sub PUCTNE 35 PY mot into
 SmaI / SphI site of pTTQ19
 clones cut E SphI / EcoRI
 5 kb 1 2 3 4 5 6 7 8 9 10

ANY 8B/55



To Page No. _____

ed & Understood by me,

Date

Invent d by

Dat

Lisha Xu

8/3/95

R corded by

CONY Longo

8/13/95

Test extension of short primers
 Δ [tag], Δ length of primer

From Page No. _____

³²P fid pri: 6 - fide Temp

" 8

" 10

" 12

" 14

" 16

(P140, 250nm primer)

Mix A

46 μ lTag μ l

set buffer 5B

.0016

.007

.04

.2

1

5

2

2

2

2

2

2

2

2

2

2

2

2

2

2

2

2

2

50 μ l

70°C

20 min

22 min

stop with

25 μ l

aqd seq

stop

solution

load

3 μ l

on

25%

(PAGE

some as P155, 7)

2000V (gets ~11mA)

for

3 hr

.0032

units

(table 4)

21 pmol

at

in

20 min

(based on units

a map

efficiency)

input primer is 1 pmol 42 mer

=

~20 pmol

at incorp

for

20 nt ssDNA seq

Page N ____

22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
----	----	----	----	----	----	----	----	----	----	----	----	----	----	----

10 nM primer Cf
1 pmol primer total / 50 μ l R_x

A diagram illustrating a sequence of three horizontal arrows pointing to the right. The first arrow is at the top, the second is in the middle, and the third is at the bottom. Each arrow is preceded by a small '2'.

A 10x10 grid with handwritten '2's forming a staircase pattern. The '2's are located at the following (row, column) coordinates: (1,2), (1,7), (2,3), (2,8), (3,4), (3,9), (4,1), (4,5), (4,10), (5,2), (5,7), (6,3), (6,8), (7,4), (7,9), (8,1), (8,5), (8,10), (9,2), (9,7), (10,3), (10,8), (10,10).

(for 40 Rxns)

utations:

Jul		
25	1	will

24			
----	--	--	--

0.0422

100847

4	2	2	2	2
---	---	---	---	---

Mix A

10X PCR buffer
50 mM MgCl₂
10 mM dNTPs

1.540 ml	✓
----------	---

200					✓
-----	--	--	--	--	---

60				✓
----	--	--	--	---

[illegible]

1240 ml

are 46 μm /K \times u

CF = 1.5 mM $MgCl_2$

255 μ M dNTPs
in Rxn.

To Page No._____

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Date

$$8 \overline{) 7195}$$

Invented by

Recorded by

Date

83-95

Maia Polay

PAGE 144 OF NOTEBOOK WAS BLANK

Result:

1. primer length 10 and longer and extended by Tag.
2. will test Tne vs Tag next to see if Tne does better than Tag

Used & Understood by me,

Polamp

Date

8/

14/15

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Recorded by

Date

7-3-95

To Page No. _____

146

From Pag No.____

32P fid pri 6 . fidel Temp

11

1

•

1

(P/40 250 nm primer) *preheated to 70°C w/mix A*

TR diluted in SRB 7/22/95
R.L.

0.9 156 u/v

0.0625 $\frac{1}{1}$

0.25 u/l

[illegible]

Ten 2/31/95 lin

100%	5/31	✓
100%	1/1	✓

10.8156	15
2.0175	11

0.0625	0.0625
0.0625	0.0625

[illegible][illegible]

Mix A (p.143)

$V_f = 50 \mu l$

20 min 70°C, stop with 25 µl cycle seq
stop solutro

heat to 90°C , 5 min before loading

25% PATE run as P142 and 155, 7; ran 3hr 2000V

mix A p. 143 scaled up 1.5x = 2.31 mL H₂O

300ul 10x PCR buffer (from Kala)

90 μ l 50mM $MgCl_2$ (made from 1M 2/1)

60ul 10mM dNTPs v

2760 ul

↳ 50ul in m
950ul H₂O

T	Pag	N
1	1	1
2	2	2
3	3	3
4	4	4
5	5	5
6	6	6
7	7	7
8	8	8
9	9	9
10	10	10
11	11	11
12	12	12
13	13	13
14	14	14
15	15	15
16	16	16
17	17	17
18	18	18
19	19	19
20	20	20
21	21	21
22	22	22
23	23	23
24	24	24
25	25	25
26	26	26
27	27	27
28	28	28
29	29	29
30	30	30
31	31	31
32	32	32
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43	43	43
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54	54	54
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80	80	80
81	81	81
82	82	82
83	83	83
84	84	84
85	85	85
86	86	86
87	87	87
88	88	88
89	89	89
90	90	90
91	91	91
92	92	92
93	93	93
94	94	94
95	95	95
96	96	96
97	97	97
98	98	98
99	99	99
100	100	100

Witnessed & Understood by me,

Dat

Invented by

Dat

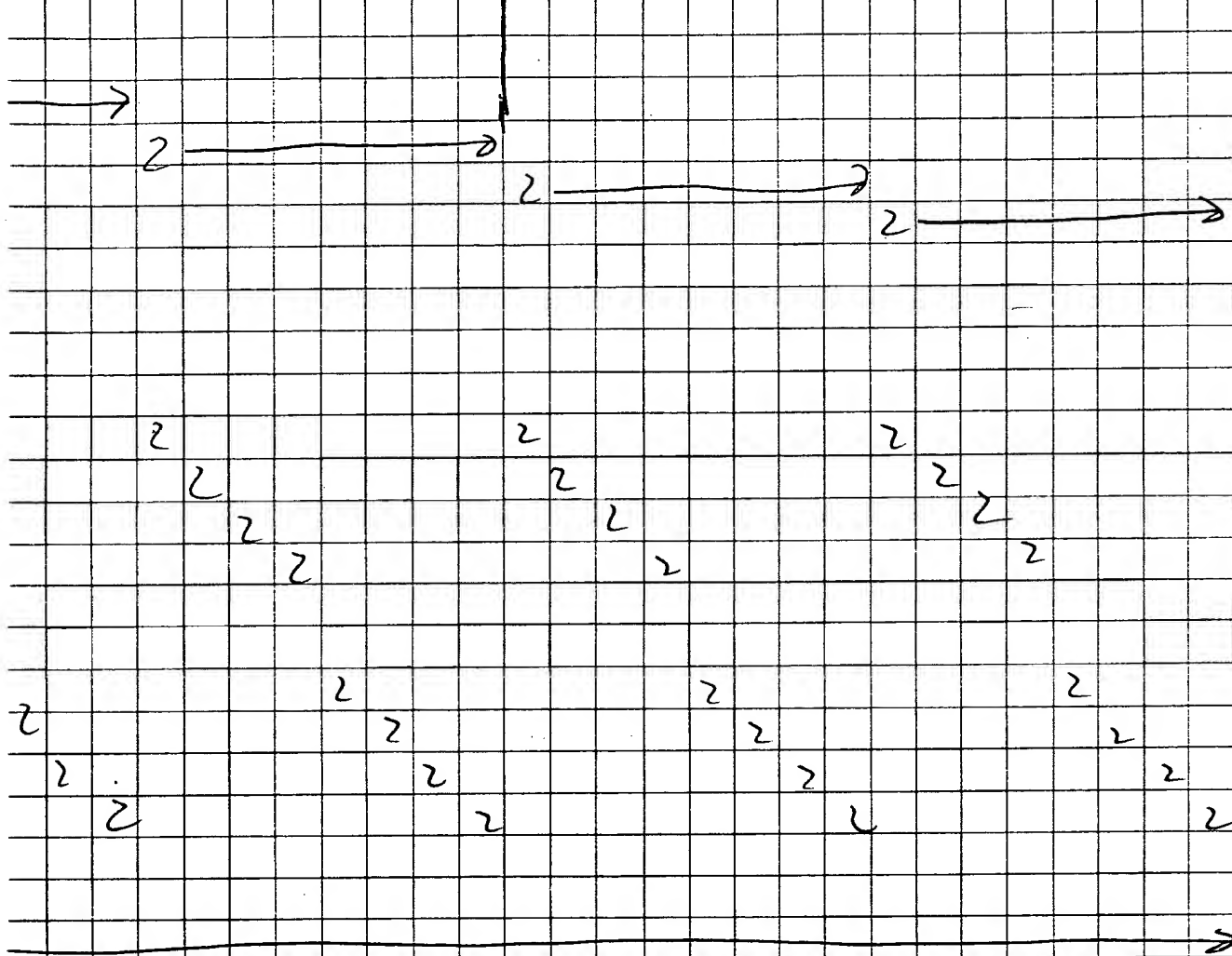
Deane Polans

8/7/95

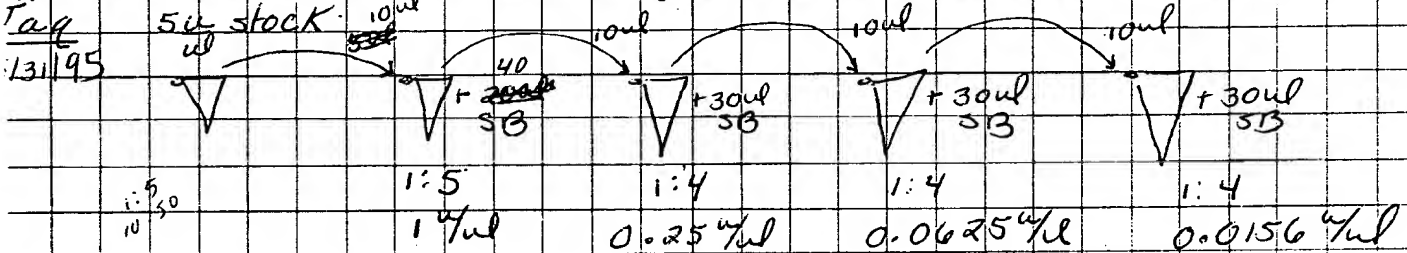
Recorded by

J-495

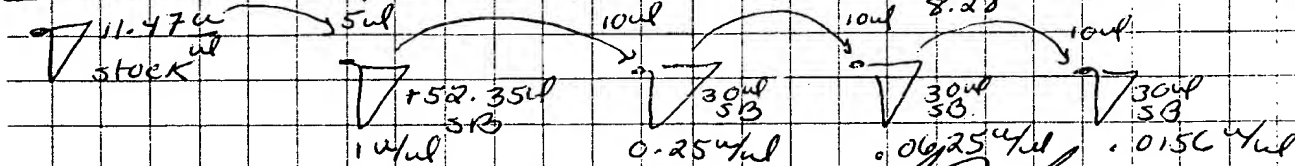
22/23/24/25/26/27/28/29/30/31/32/33/34/35/36/37/38/39/40/41/42/43/44/45/46/47/48



same dilutions: in Tag storage buffer - 12/7/94



The 1st normalize to Tag units p 127 19 x 5 = 11.47 u/l (Tag was thought to be 5 u/l)



To Pag No. _____

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Date

Inv nted by

Date

Michael Polak

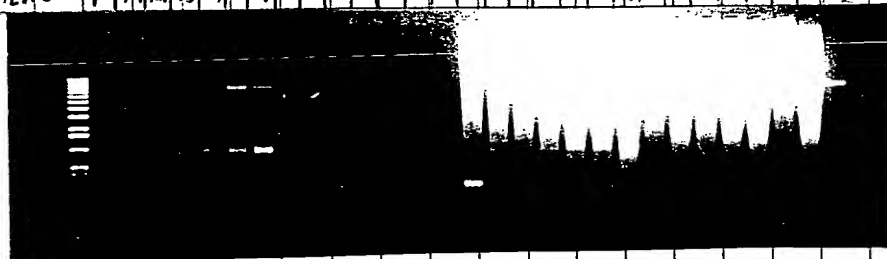
8/7/95

Recorded by

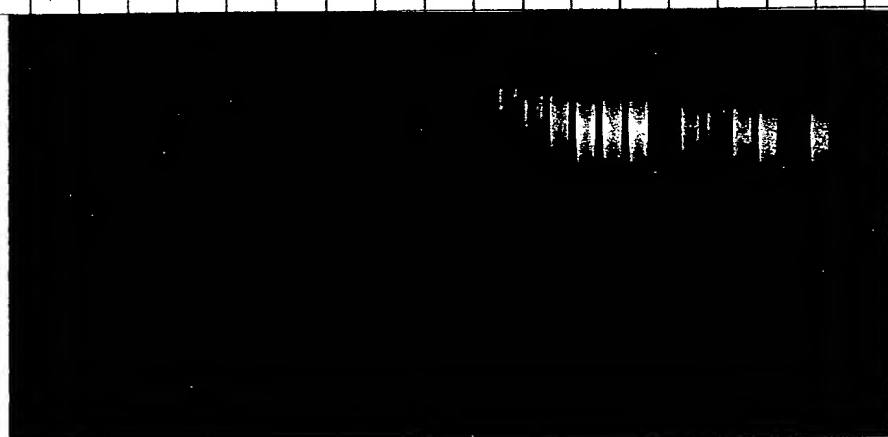
Paulyn

P-4-SJT

^{15}O template \rightarrow $1\mu\text{Taq} : 1\text{mU Tne}$ $1\mu\text{Taq} : 10\text{mU Tne}$
 $+$ $-$ $+$ $-$
 $[\text{MgOAc}]_{\text{mM}}$ 1 1.1 1.2 1.3 1.4 1.5 1.6 1 1.1 1.2 1.3 1.4 1.5 1.6 1 1.1 1.2 1.3 1.4 1.5 1.6 \leftarrow $1\mu\text{Taq} : 2\text{mU Tne}$
 $1/10\text{mM Mg}^{2+}$



815145



8/5/95

i) primer / target dependent nonspecific products, which are seen with both μ unit and μ units Tne. The products are discrete bands, mostly ≤ 1 Kb. The ~~total~~ yield of these nonspecific products, as well as the specific 7.5 Kb product increase as $[Mg^{2+}]$ increases. The highest ratio of specific to nonspecific product occurs at 1.5 mM Mg .

2) primer/target independent products. The products form an intense smear from wells down to ≈ 200 bp. As $[Mg^{2+}]$ increases, the size of products in smear decreases and the smear becomes more intense. This is the same Mg^{2+} effect that was observed in a short PCR with Tne alone.

To Page No.

earlier Poling

8/7/95-

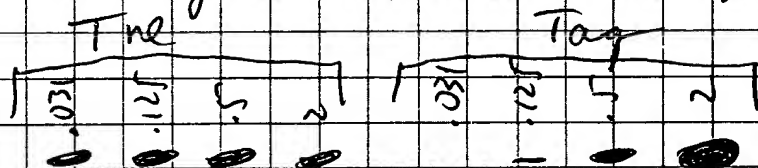
Recorded by

Recorded by *Carolyn Lamb*

8/5/95

Data on P 151 152

ii. In 16mer primer, T₂ again shows first appearance of ~~smear~~ 'smear' but T₂ has greater yield at 12 units of enzyme.



less Tne is needed to extend a primer.
Maybe Tne is more processive
That is consistent with Tag giving go
extensive (equal or better than Tne) at high
and ~~constant~~ could explain how Equil unit
of Tag, Tne for DNase I treated gaps
DNA act differently for primer extension.

ag N — see p. 140

nd-label Fid 16: 4 μ l 32 P γ ATP ref 8/4/95
48.08 μ l H₂O ✓4 μ l 5x Kinase buffer ✓3.32 μ l 10 μ M Fid 16 oligo (33.2 μ M, 4.46 pmol/run)4 μ l 32 P γ ATP ref 8/4/95 (3.33 μ M, 4.46 pmol/run)0.6 μ l PNK20 μ l in 9600 tube

37°C, 30 min ✓

55°C, 5 min ✓

cool to ~4°C ✓

+ 4 μ l Fidel Temp, 10 μ M 42mer (40 pmol)

80°C, 5 min

cool for 15 min to RT in PCR machine

+ 129.4 μ l 10 mM Tris pH 8.0

store at -20°C

To Page No. _____

sed & Understood by me,

J Polay

Date

8/14/95

Invented by

Recorded by

Cawlyn Comb

Date

8/7/95

PAGES 150-151 OF NOTEBOOK WERE BLANK

32p 42 mer for 3' exo assay
of 3' exo (-) Klenow fragment

Project N. _____ Exhibit L-151
Book No. _____ Appl. No. 09/558,421

61

ag N. _____

Endic Template 10 μ M (42 mer)	5.8 μ l	✓	5 μ mol total
3-P-ATP	7	✓	
5x Klenow buffer	7	✓	
PNK 1 μ l	2	✓	
H ₂ O	13.2 μ l	✓	
	35		

37°C, 30'

70°C, 5'

1 M KPO₄ pH 7.6

1 M Kmonobasic 1.3 ml

1 M K dibasic 8.7 ml

VP = 10 ml

To Page No. _____

Read & Understood by me,

Researcher a Polak

Date

8/14/95

Invented by

Recorded by

Date

8-8-95

Project No. _____

Book No. _____

TITLE Primer extension: 4 [eng], Tag vs Tne
time course with
Fid 16 primer

From Page No. _____

purpose: In the last primer extension experiment, ~4x less Tne than Tag was able to extend the Fid 16 primer to a full length 42mer product. p. 150. The ^{highest} level of Tag (2u), that was tested, made even more full-length product than the highest level of Tne tested (2u). ~~Today's expt.~~ The purpose of today's expt. is to confirm the results of p. 150 and to determine whether Tne & Tag have different affinities for DNA/primer binding or if they are the same but Tne is better at extending after a stop. ^{the time course & no eng run will reveal if Tne causes a stop} Sequencing rxns and the Fid 16 primer without enzyme will also be run on the 25% gel.

materials:PCR/Mg²⁺ mix to mix with enzyme prior to beginning rxns:

	conc
25 uL 10x PCR mix ✓	1.25x
7.5 uL 50mM MgCl ₂ ✓	1.25x
167.5 uL H ₂ O ✓	
<u>200 uL</u>	

2 use 16 uL ✓

+ 4 uL of eng. dilution in 5B ✓

20 uL - 1x PCR buffer = 20mM Tris 8.4, 50mM
tubes 1-8 1x MgCl₂ CF = 1.5mM

mix A for 10 rxns:

80 uL 10x PCR buffer ✓
63 uL H ₂ O ✓
24 uL 50mM MgCl ₂ ✓
20 uL 10mM dNTPs ✓
40 uL ³² P Fid 16 annealed to Fid 1 Temp p. 14
<u>800 uL</u>

Keep at 70°C

enzyme dilutions in Tag storage buffer: same as on p. 147
same preps of Tag & Tne

5 uL STOP soln. from cycle sequencing kit in 9600 tubes 1-40

T Page N

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Date

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Dat

Polamp

8/

11/19/95

R corded by

Paula L. Paul

8/8/95

ag N —

	1	2	3	4	5	6	7	8
tubes	1-5	6-10	11-15	16-20	21-25	26-30	31-35	36-40
2/95	1' 2' 5' 10' 20'	all prewarmed to 70°C						
1 Tne mix	20							
15 PCR								
25 mg 2+								
1 Tag mix	20							
1/2 Tne mix		20						
1/2 Tag mix			20					
15 1/2 Tne mix				20				
15 1/2 Tag mix					20			
15 1/2 Tne mix						20		
15 1/2 Tag mix							20	
+ rxns w/ 30ul of mix A prewarmed to 70°C, triterate w/ p200								
move 10ul of rxn to a 9600 tube containing 5ul STOP at 1min, 2min, 5min, 10min, 20min - on ice								
at to 80°C, 5min before loading 1.5ul on 25% gel								
ter of loading: on 25% urea gel								
left	ATGC noeng	31-40	21-30	11-20	1-10	8 ATP		
	Fid16					diluted 10X		
	16ul 1.25X PCR buffer					to 1x10 ³ 3ul		
	4ul storage buffer					+ 3ul		
	+ 80ul mix A					+ 2ul H ₂ O		
	removed 10ul +					+ 1ul STOP		
	5ul stop - from cycle sequencing kit					load 1.5ul		
ded on 8% gel in same order								
To Page No.								

ed & Understood by me,

Date _____

Inv nted by

Dat

Polanco

3

Recorded by

8/8/95

To Page No. 13

From Page No. _____

10 Rows

Tm PCR
conditionBSA 20mg/L
1M KH_2PO_4 pH 7.6

✓

A
2.5 μL

B

1)

RSA-O.T.

5

10X PCR buff

✓

50

50 mM MgCl_2

✓

70

70 mg

50% sterile glycerol

✓

140

1)

H₂O (sterile)

✓

232.5

350

3:42 min

✓

10

10

(fidel Template P61

* see P 74-75
* Berketon Ockerson

480

480

3:42 min

nr
arr

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

(A)

48 μL →

(B)

48 μL →Klenow exo-
wt CK04 175 μg dil 25 μL 5 μL 1 μL

Klenow exo(-)

wt EJIP4 130 μg dil 25 μL 5 μL 1 μL

Klenow (+) exo

0.6 μL

0.006

0.006

dilutions
with T₁
dil buff

start 1:2

3 hr

25 μL cycle
stop solload 3 μL
8% PA

Witness d & Und rst od by me,

Polamp

Dat

8/14/95

Inv nted by

R c rded by

Dat

8-9-95

To Pag N

From: Hartman, Chris
 T : Lasken, Roger
 C : Rashtchian, Ayoub
 Subject: Exo minus Klenow
 Date: Monday, August 07, 1995 3:40PM
 Pri rity: High

roj ct No. _____
 Test No. _____

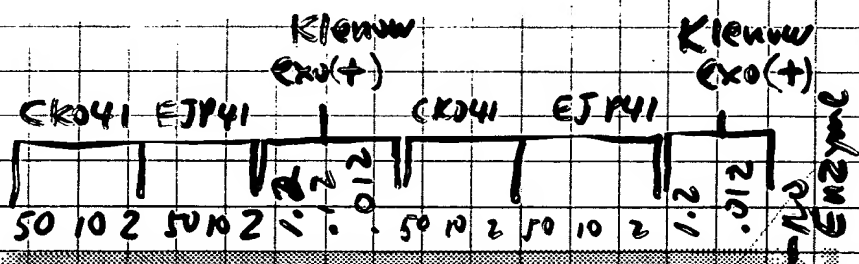
P63, 10
 63

Pecton
 Dickinson
 Conditions

Terg
 PERL
 buffer

Roger, The unit values for the bulk exo minus klenow are as follows.

Lot No. U/ul
 CK041 75
 EJP41 130



P42 mer

To Page No. _____

s d & Understood by m , <i>Polay</i>	Date 8/14/95	Invent d by <i>[Signature]</i>	Date 8-14-95
	Recorded by <i>[Signature]</i>		

From Pag No. _____

33 correct (p138, 9)
20 μ M4.38
✓ 2.18 μ l87
~~43.0~~ p
hr

M13 mp19 s DNA (+)

✓ 200 μ l21.8 pmol
total

1M Tris pH 7.5

✓ 10.6 μ l✓ 212.78 μ l50 mM
Tris(0.0 pmol
long)0.1025 pmol circle / μ l \Rightarrow 743 pmol nt / μ l
use 2 μ l / 50 μ l Rxn for 1.5 nmol nt / Rxn

Mix A

(33.4 Rxns)

Taps MgCl₂ KCl
(of p120, 9)(add 15 μ l
next time)103 μ l ✓320 μ l ATP 10mCi/ml 300 μ l pmol

3 ✓

+ ATP 10mM

33.4 ✓

(200 μ M)

+ CTP 10mM

33.4 ✓

+ GTP 10mM

33.4 ✓

+ T₀ 5 μ l66.8 μ l ✓

33. mp19

66.8 μ l ✓743 pmol nt / μ lH₂O

1.163 ml ✓

1.503 ml

1.0 μ l / 50
1.5 nmol ntuse 45 μ l / 50 μ l Rxn

T Pag

With ss d & Understo d by m ,

S. Polansky

Dat

8/14/95

Invented by

Rec rd d by

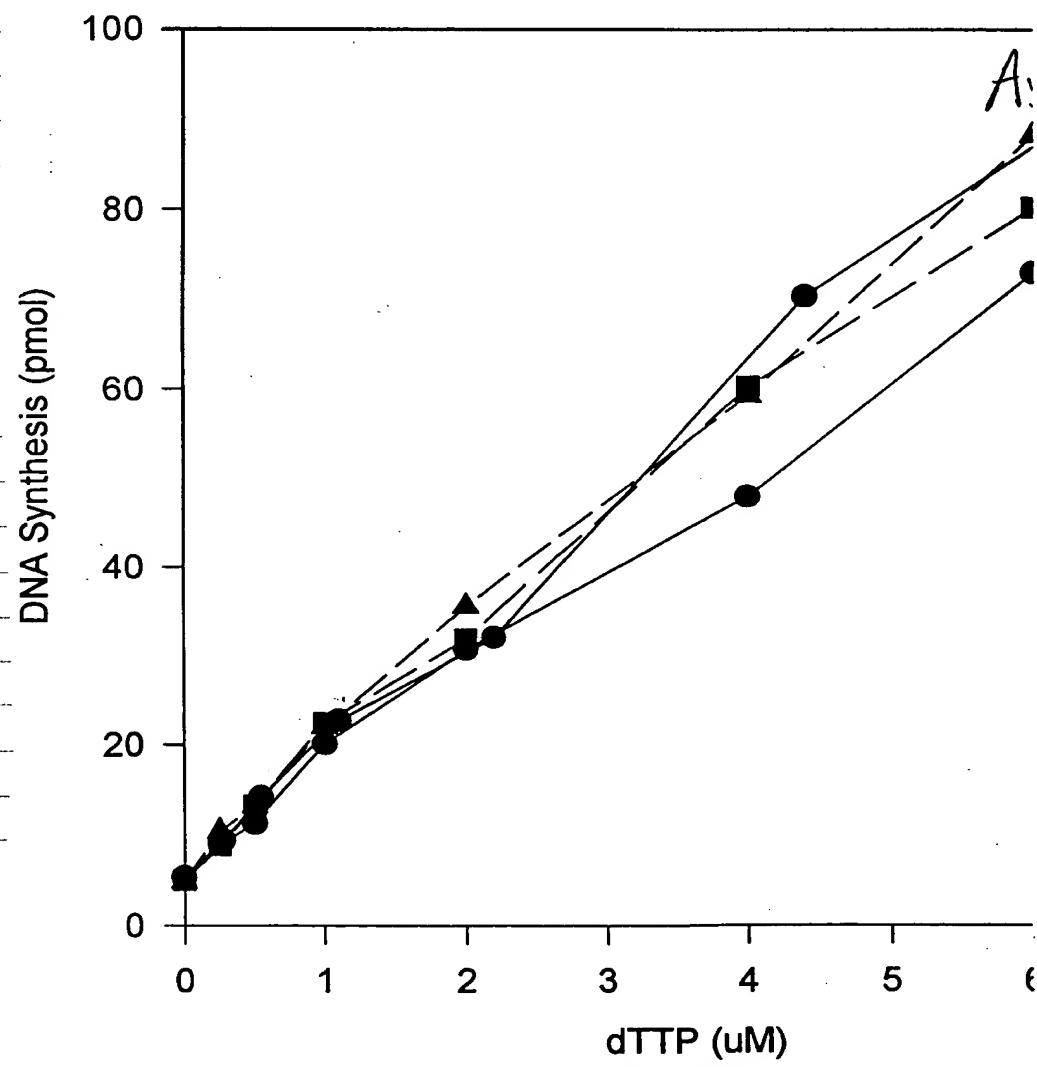
Dat

F. S. J.

Recorded by

Project No. <u>5700</u>		Total dTTP <u>0.125</u>		TITLE <u>Results P65</u>	
Book No. <u>(2.1)</u>		dpmol		assay	
0	82.60	5.4	0	12.5	11%
0.25	137.80	9.1	12.5	25	11%
0.5	172.00	11.4	25	50	10
1	303.80	20.1	50	100	7.5
2	463.40	30.7	100	200	6
4	725.00	48	200	300	6
6	1103.80	73	300		
0	76.00	5.0			
0.25	134.40	8.9			
0.5	201.00	13.3			
1	339.20	22.4			
2	482.00	31.9			
4	910.20	60.3			
6	1211.80	80.2			
0	82.20	5.4			
0.25	145.80	9.1			
0.5	216.20	14.3			
1	344.60	22.8			
2	485.00	32.1			
4	1062.00	70.3			
6	1408.00	93.2			
0	74.00	4.9			
0.25	158.40	10.5			
0.5	201.00	13.3			
1	332.60	22			
2	538.20	35.6			
4	896.00	59.3			
6	1333.20	78.3			
0	57.20				
0.25	72558.92				

200 uM dATP, dGTP, dCTP



Processivity of Tag, Tne, and Ultima

Tag N _____

extension of 33-mer correct primer annealed to m13mp19 ssDNA
 serial enz dilutions, 2 min extension and 10 min endpt extension
 2 units - 0.0078 units, in 50ul rxns, reactions started w/ 2ul enz.

1/9/95

action cocktail for 35 rxns = 175ul 10x PCR buffer
 1347.5ul H₂O

ote: 42 ³²P primer: 1 m13 circle 52.5ul 50mM MgCl₂

* mistake, see 8/11/95 35ul 10mM dNTPS

were 2ul of the labeled 70ul ³²P-33mer correct annealed

* annealed primer was
 diluted w/ 70ul m13

1680ul

to m13mp19 - the Kinase
 rxn was done as on

p. 12 NB10, then 46ul

of m13 ssDNA added

0.26ug/ul m13 stock

" 32 P33 correct m13 "

this was 42 pri / circle - self 1/64
 where more m13 added to get 1 pri / circle

zyme dilutions in Tag SB

Tag, 5/31/95, 5ul

50ul

4ul

16ul SB

10ul

10ul

10ul

10ul

10ul

10ul

10ul

10ul

10ul

10ul

1:5

1ul

1:2

0.5ul

0.25ul

0.125

0.0625

0.0313

0.0156

0.0078, 0.0039ul

mitarray 7/30

Tne 7/31/95 11.47 ul - this value is normalized to Tag p. 147

5ul

11.47ul

10ul

52.35ul SB

1:11.47

= 8.1ul

then, serial dilutions made in the
 same way as for Tag

Ultima

6ul

10ul

10ul

20ul SB

10ul

10ul

10ul

10ul

10ul

10ul

10ul

10ul

10ul

Lot 0643 12/31/95, Perkin Elmer

same dilutions as for Tag & Tne, but
 Ultima units are not normalized to
 Tag units

To Page No. _____

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Polamp

Date

8/14/95

Invent d by

Recorded by

Con

Dat

8/9/95

From Page N. _____

- ~~25ul~~ 48ul mix in a 9600 PCR tube, preheated to 70°C
- reactions were started by adding 2ul of enzy w/ P2 and triterating w/ P200
- after 2 min at 70°C, in 9600, rxns were stopped w/ 25ul Cycle sequencing Stop solution and kept at -20°C overnight prior to loading on 8% gel

Tubes	1 — 10	11 — 20	21 — 30
	Tag	Tne	Tma
	0.0078 → 20 units	0.0078 → 20 units	0.0078 → 20 units

The 20 units rxns were incubated for ¹⁰min; while all the other rxns were incubated for 2 min

T Pag N.

Withn ssed & Und rst d by m ,

Dobolamp

Dat

8/14/95

Inv nt d by

R corded by

Daulm Rom

Dat

8/9/95

Project No. _____

Book No. _____

TITLE Extension of 16-mer by Tag + Inc
with Δ MgCl₂ + Δ KCl

158

From Page No. _____

2

general overview of conditions tested:

fix MgCl₂

1.05 mM

1.5 mM

Δ KCl (mM)

0 25 50 85

0 25 50 85

each condition tested

w/ 0.0312 μ Tag + Inc

2.0 μ Tag + Inc

in 50 μ l rxns at:

for 20 min

fix KCl

50 mM

Δ MgCl₂ (mM)

1, 1.2, 1.5

= 44 rxns

for 1, 50 μ l rxn: 1 μ l 1M Tris ^{8.5} ~~8.4~~ CF = 20 mM * note that real pCK
41.08 μ l H₂O buffer is pH 8.4

1.42 μ l 3M KCl for 85 mM CF

1.5 μ l 50 mM MgCl₂ for 1.5 mM CF

1 μ l 10 mM dNTP CF = 200 μ M

* 2 μ l ³²P 16mer on Fide Temp CF = 10 nM

2 μ l enz to start rxn

50 μ l

* End-label 16mer as on p. 149

mix A for 50 rxns: 50 μ l 1M Tris, pH 8.5 ✓ 22.5 ✓
18.75 μ l H₂O ✓ 843.75 ✓

50 μ l 10 mM dNTP ✓ 210.5 μ l 705 μ l 370 μ l 22.5 ✓

100 μ l ³²P 16mer annealed to Fide Temp (✓)

20.75 μ l

T Page 1

Witnessed & Understood by me,

Date

Initiated by

Date

DD Polanco

8/14/95

Recorded by
Davidson Pomh

8/10/95

ig N _____

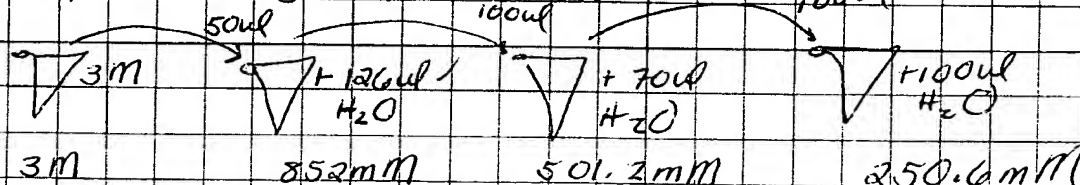
the expt w/ varied KCl

B w/ 10.05 mM MgCl₂

7 runs

705.5 μ l A17.85 μ l 50 mM MgCl₂ ✓✓7.65 μ l H₂O ✓✓731 μ l2 runs 180.6 μ l B C_f = 0 mM KCl
+ 21 μ l H₂O → 48 μ l / run
4 tubes 2 runs = 4180.6 μ l B+ 21 μ l 250.6 mM KCl C_f = 25 mM KCl / run180.6 μ l B+ 21 μ l 501.2 mM KCl C_f = 50 mM KCl180.6 μ l B+ 21 μ l 852 mM KCl C_f = 85 mMC w/ 10.5 mM MgCl₂705.5 μ l A25.5 μ l 50 mM MgCl₂ ✓731 μ lsame but use
180.6 μ l C for each

Serial dilution of 3M KCl stock: ✓



To Page No. _____

d & Understood by m ,

Date

8

Invented by

Date

Recorded by

Eunhyun Kim

8/10/95

Polcup

From Page No. _____

for expt w/ varied $[Mg^{2+}]$:

for 14 rxns

D

= 581 μ l A11.62 μ l 3M KCl ✓9.38 μ l H_2O ✓

C = 50 mM KCl / rxn

for 4.2 rxns

602 μ l180.6 μ l D+ 21 μ l 10 mM Mg^{2+} /use 48 μ l / rxnC = 1 mM Mg^{2+} / rxn180.6 μ l D+ 21 μ l 12 mM Mg ✓C = 1.2 mM Mg / rxn180.6 μ l D+ 21 μ l 15 mM Mg^{2+} C = 1.5 mM Mg dil of 50 mM $MgCl$ stock:

50 mM

50 μ l+ 116.5 μ l H_2O

15 mM

100 μ l+ 25 μ l H_2O

12 mM

100 μ l+ 20 μ l H_2O

10 mM

Enzyme dilutions in Taq storage buffer: ^(SB)Taq, 5/31/95 stock 5 μ l (not real units)5 μ l+ 40 μ l SB14 μ l10 μ l+ 631 μ l SB0.0156 μ lTne, 7/22/95 stock = 11.47 μ l, normalized to Taq p. 127 + 14711.47 μ l5 μ l+ 52.33 μ l SB14 μ l10 μ l+ 631 μ l SB0.0156 μ l

With ss d & Und rst d by m ,

Dat

8/14/95

Inv nt d by

R cord d by

J. J. J. J. J.

Dat

8/10/95

T Pag 1

age N _____

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Mg ²⁺ OKel	48				48	48			48				48												
Mg 25 Kel	48				(48)				48				48												
Mg 50 Kel		48				48				48					48										
Mg 85 Kel				48			48				48					48									
Mg OKel																	48				48				
Mg 25 Kel																	48					48			
Mg 50 Kel																		48					48		
Mg 85 Kel																			48					48	
1 Mg																									
1.2 Mg																									
1.5 Mg																									
50% 4ul Tne																									
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ss d & Understood by me,

Date _____

Invented by**Date**

Polans

8/14/95

Recorded by

To Page No

Project No. _____

Book No. _____

TITLE _____

From Page No. _____

	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
		✓			✓				✓			✓			✓			✓	
.5mg OKel	48				48														
.25mg OKel	48				48														
.50mg OKel		48			48	48													
.35mg OKel			48		48	48	48												
50mg OKel									48			48			48			48	
1.2mg									48			48			48			48	
1.5mg										48			48			48			
.0156 Tne									2	2	2								
.0156 Taq												2	2	2					
1 Tne	2	2	2	2											2	2	2		
1 Taq					2	2	2	2										2	2

200V, 40mA 12 PM -
12:50 PM

T Pag N

With ssed & Und rst d by m ,

Date

Inv nt d by

Dat

D. Polansky

8/14/95

Record d by

D. Polansky

8/10/95

Redid processivity of P155
 expect expand slower (pol7ran)

From Page No. _____

and correct error in pri / ~~per~~
 from 42 to (here) 30

32P 32 correct. m p19
 P155

2 μ lm p19 0.26 μ g / λ

70
 70 μ l

2.6 pmol circ
 total
 now have
 pri / circle = 1

10 x PCR buffer
 H₂O
 50 mM MgCl₂
 10 mM dNTP

mix A

70 μ l
 175 μ l
 1347.5 μ l
 52.5 μ l
 35

VF = 1670 μ l

33. m p19

~ 0.22 pmol circ
 per 50 μ l rxn

(preheat to 70°C
 47 μ l mix A + 2 μ l of pol
 to start \rightarrow kill with 25 μ l
 cycle seq stop sol

same (enz) as p. 155 and 5 more 2 fold dilutions

Tube 15
 20 units / 50 λ rxn

Tube 14

2 1 .5 .25 .125 .063 .031

Tag = 1-15

.0156 .0078 .0038 0.00194 0.000

Tne = 16-29

.000484 .000242 = tube 1

From: Hartman, Chris
To: Lasken, Roger
Subject: Rashtchian, Ayoub
Date: Exo minus Klenow
Priority: Monday, August 07, 1995 3:40PM
High

Project No. — Exhibit L-157
Book No. — Appl. No. 09/558,421

P6310
63

*Becton
Dickins n
Conditions*

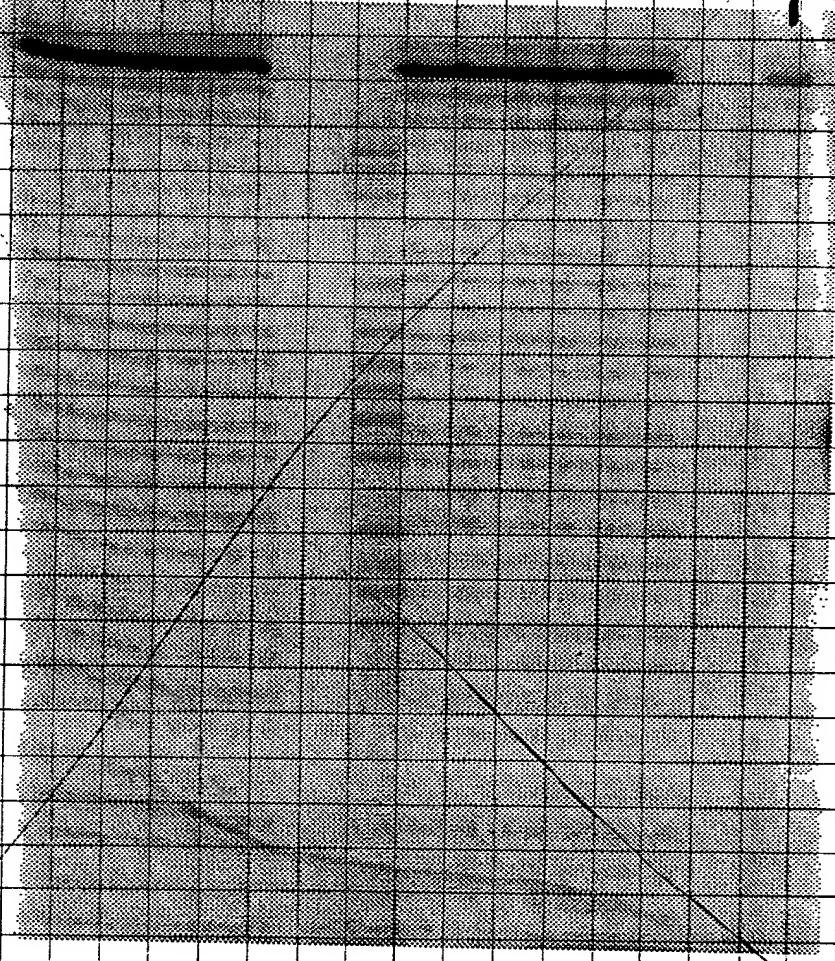
*Tag
PCR
buffer*

Roger, The unit values for the bulk exo minus klenow are as follows:

Lot No.	U/ul
CK041	75
EJP41	130

Klenow exo(+)						Klenow exo(+)					
CK041			EJP41			CK041			EJP41		
50	10	2	50	10	2	50	10	2	50	10	2
<i>1.2</i>						<i>1.2</i>					

P42 mer



Read & Understood by me,
Polamp

Date
8/14/95

Invented by
[Signature]
Recorded by

Date
8-14-95

To Page No. —

Test supernix for (JATP)
[JATP] [JCTP]

68

From Page No. _____

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24

67 ① (-JATP)
② (-JCTP)
③ (-JCTP)

4 JATPs

2.5 μ m
5 μ m
10 μ m
20 μ m
40 μ m
60 μ m

* Mix B #11

2.5
5
10
20
40
60

H₂O5
50 μ l

- assemble on ice
- put in 2600 4°C

sample to 70°C \Rightarrow 45"
sample to 4°C

* for B
its 220 μ m
(at 1.1x)
30 μ l B
50 μ l H₂O
4-110 μ l

Witness d & Understood by m ,

JOP oiang

Dat

8/21/95

Invent d by

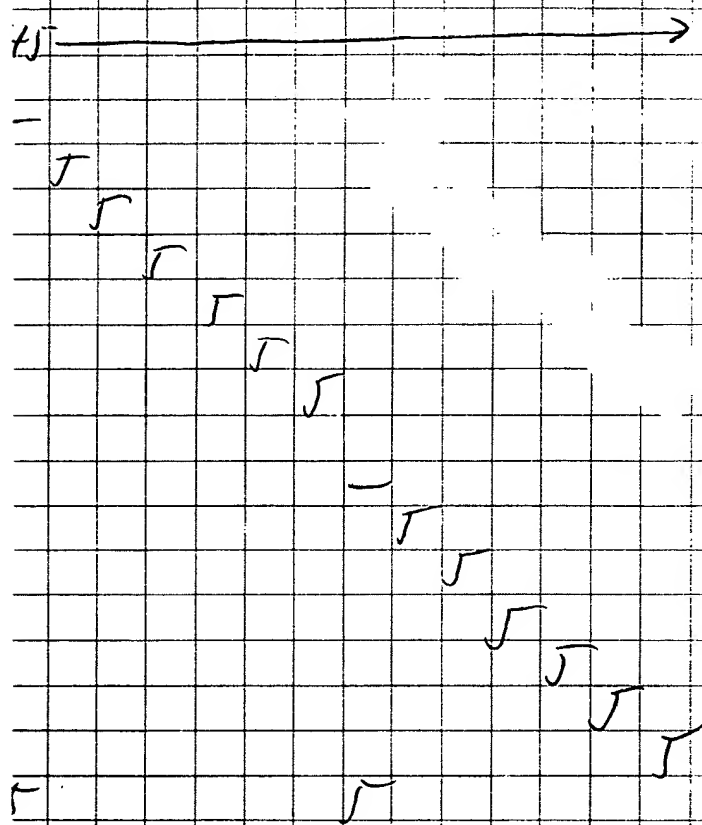
R c rded by

Dat

8-15-95

T Pag 1

20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42



43 is mix (1) ice only
ice no 70°C before EOTF

44 is mix (3) ice only

45 2# Rxn # 15 }
46 2# Rxn # 29 } no TCA

To Page No. _____

Read & Understood by me,

Polamp

Date

8/21/95

Invested by

[Signature]

Date

8-11-95

Recorded by

Project No. _____

Book No. _____

TITLE _____

Route P 65

70

From Page N . . .

1 103.00
2 264.00
3 275.00
4 638.00
5 1025.00
6 1248.00
7 2048.00
8 56.00
9 204.00
10 297.00
11 619.00
12 1011.00
13 1430.00
14 1879.00
15 94.00
16 488.00
17 653.00
18 1332.00
19 2200.00
20 3902.00
21 5706.00
22 83.00
23 457.00
24 643.00
25 1289.00
26 2218.00
27 4406.00
28 4082.00
29 53.00
30 57231.00

pmsol

3.5
148
15.5
41
68
74
145
2.2
11
17
40
67
96
128
2.9
30
42
89
150
273
385
2.1
28
41
86
151
304
282

42.9 cpm pmsol

1 768.00
2 854.00
3 926.00
4 928.00
5 979.00
6 1443.00
7 1721.00
8 818.00
9 763.00
10 971.00
11 931.00
12 1155.00
13 1230.00
14 1473.00
15 16.00
16 739.00
17 14195.00

8.1
32
53
53
68
198
277
22
6.8
65
54
117
138
207

not very high? HJATP BK6D

(14195 cpm)

(50 pLRx + 10 pLR EDTA)

(2 x spotted)

(40000 pmsol at 75x)

= 10.64 CH
To Pag

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note - JTP is on P66, 67

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TITLE Determination of how to kill DNase

166

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DNase I will be used to treat the enz prep. If DNA contamination is the cause ^(primer dimers) of the smear, then the DNase I treatment may eliminate the smear. 1st we'll establish how to kill DNase I, after it has been mixed w/ Tne, so it won't be active during a

1. DNase I rxn will be killed w/ CDTA + heat for 5, 10 + 30 min.
A second DNase I rxn will be killed ~~only~~ by heat only for 5, 10, 30.
After the killing treatment, the rxn will be mixed with QX174 RF.
If the DNase I was killed, the QX174 won't be degraded, even after a 3hr incubation.

materials: QX174, 0.25 μ g/ μ l in 0.1 mM CDTA from LTI Lot FA370.

DNase I, 1 μ g/ μ l in SB = 20 mM NaOAc pH 6.5
5 mM CaCl₂
50% glycerol

25 mM CDTA - 50 μ l 0.5 M CDTA pH 8
950 μ l H₂O

200 mM Tris 8.5 (note: the DNase I buffer is 8.4)
200 μ l 1 M Tris 8.5
800 μ l H₂O

20 mM MgCl₂ - 200 μ l 50 mM MgCl₂
+ 300 μ l H₂O

200 mM KCl - 200 μ l 60.7 μ l 3 M KCl
+ 933.3 μ l H₂O

10X DNase I buffer = 200 mM Tris-HCl pH 8.4
LTI 20 mM MgCl₂
Lot GK2410 500 mM KCl - it was a bit bubbly after mixing

0.8% TAE agarose gel w/ EtBr

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D. Polak

8/11/95

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8/15/95

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DNase I rxns: in 9600 PCR tubes

1) 14 μ l H_2O
 + 2 μ l 10x DNase I buffer
 + 4 μ l DNase I, 14 μ l $C_F = 0.2 \mu$ l
 20 μ l
 Kill with
 heat + CDTA

(2) 14 μ l H_2O
 2 μ l 10x DNase I buffer
 4 μ l DNase I $C_F = 0.2 \mu$ l
 20 μ l - only heat kill this rxn

15' at $RT (= 23^\circ C)$ → during this time 1 μ g
 DNA should be digested

remove 4 μ l (= 0.8 units DNase I)
 + 4 μ l 0.25 μ g/ μ l ϕ X174
 + 1.6 μ l 10x DNase I buffer
 + 10.4 μ l H_2O
 20 μ l w/ 1 μ g

(series to see how long it takes active
 DNase I to degrade 1 μ g ϕ X174)

→ immediately remove 4 μ l + 1 μ l 10x loading dye w/
 100 mM CDTA = 0' Kill time
 0 time incubation w/ ϕ X174
 → 2' later remove 4 μ l + 1 μ l LD = 0' Kill time
 2' w/ ϕ X174
 → 15' later remove 4 μ l + 1 μ l LD = 0' Kill time
 15' w/ ϕ X174
 → 1 hr later remove 4 μ l + 1 μ l LD = 0' Kill time
 1 hr w/ ϕ X174
 → 3 hr later remove 4 μ l + 1 μ l LD = 0' Kill time
 3 hr w/ ϕ X174

⑦ Kill the remaining 16 μ l w/ 1.36 μ l 25 mM CDTA $C_F = 2$ mM
 ①+② heat to $75^\circ C$ in 9600

→ 5' of heat kill, remove 4 μ l
 for rxn ①

+ 4 μ l ϕ X174
 + 4 μ l 200 mM KCl $C_F = 50$ mM, 16 μ l
 + 1.6 μ l 200 mM Tris 8.5 $C_F = 20$ mM, 16 μ l
 + 2.02 μ l 20 mM $MgCl_2$ $C_F = 2.02$ mM
 + 4.4 μ l H_2O
 20 μ l → 3 hr at RT
 for 20 μ l

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remove 4ul aliquots and treat in the same way after 15' and 30' Killing with heat and EDTA

for rxn ②

for the DNase I rxn that was killed by only heat, take 5', 15', and 30' killing time points by removing 4ul of rxn to a tube w/

4ul ϕ X174, 0.25 μ g/ μ l
4ul 200mM KCl
10.6ul 200mM Tris 8.5
10.6ul 20mM MgCl₂
4.8ul H₂O
20ul

- 3hr incubation at RT
- + 3ul 10x Loading dye w/ 100% EDTA
- run 23ul on 0.8% gel

gel order

14 wells

1Kb ladder 10ug	1ug ϕ X174	5' heat + EDTA	5' heat only	15' heat + EDTA	15' heat only	30' heat + EDTA	30' heat only	OK!! 10 ϕ X174	OK!! 2ul	OK!! 15ul	OK!! 1hr w/ ϕ X174	OK!! 3hr w/ ϕ X174	1Kb ladder
--------------------	-----------------	-------------------	-----------------	--------------------	------------------	--------------------	------------------	------------------------	-------------	--------------	----------------------------	----------------------------	------------

active DNase I
incubated w/ 1ug ϕ X174
varying amounts of time

T Pag N

With ss d & Und rst od by m ,

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Paula P. Smith

Dat

8/15/95

with assay for -20°C sample
from J80les and also 1.1X mix sum
as P 34, 52, 80 (with assay P 18)

From Page No. _____

From Page No. =

(note -20°C #11 array)
(on P 18)

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

-20 #1E 27/1

2 2 2

-20 #4E 27/1

2 2 2

-20 #7E 27/1

2 2 2

1.1X J8-95

2 2 2

1.1X Field Test
(old on P 34)

2 2 2

rTag 1/25 did
J-30-95

2 2 2 2 2

Tag Rem in
P120, 8

47% —————→

relative
to Tag

P1225
(time 0)

cpm

w/ml

w%

-20 1E

25 5911.00

26 6883.00

27 6801.00

6531 .03 .037 81%

see p 53
whole #11
of 27/1

-20 4E

28 5982.00

29 5759.00

30 5205.00

5649 .026 .029 90%

54% and
renew

-20 7E

31 6079.00

32 5062.00

33 7422.00

6187 .028 .033 85%

80% #1E 4
show better

1.1X
J-8-95

34 4974.00

35 4594.00

36 4752.00

4773 $\frac{4773}{8686} = .04 = .022$ $\frac{.022}{.023(P34)} = 96\%$

1.1X
old Field
Test

37 4389.00

38 4552.00

39 4971.00

4637 $\frac{4637}{8686} = .0213 \Rightarrow 93\%$

rTag

40 8930.00

41 8601.00

42 8299.00

8686 and .04

(by
definition)

GK60

43 8980.00

44 8618.00

45 78.00

2X
mix

46 100897.00

47 102480.00

48 102152.00

see P 53
where #11
of 27/1
54% and
review
% #1E4
show better

To Page 1

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8/21/95

Rec'd by

8-11-95

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specific activity of A mix = $\frac{121843 \text{ cpm}}{40,000} \left(\frac{50 \mu\text{l}}{2 \mu\text{l}} \right) = 63.7 \text{ cpm/pmol nt}$

$$\text{pmole} = \frac{\text{cpm}}{5 \cdot A} \left(\frac{100}{20} \right) (\text{pmol})$$

SAM CPM1

8/17/95
cc

1	3072.00	-147
2	1533.00	-72
3	1127.00	-53
4	516.00	-24
5	198.00	-9.32
6	3423.00	-141
7	1581.00	-74.5
8	1174.00	-55
9	475.00	-22.4
10	249.00	-11.7
11	3178.00	-150
12	2007.00	-95
13	2979.00	-140
14	2332.00	-110
15	2799.00	-132
16	2601.00	-122
17	2954.00	-139
18	2798.00	-132
19	3532.00	-166
20	1251.00	-58.9
21	280.00	-13.2
22	3472.00	-164
23	2974.00	-140
24	2605.00	-123

The dies at 90°C, even if the activity is only 5% of the original activity, EDTA is present (ie free Mg²⁺) Therefore, must kill DNase I at 90°C. The dies a little at 75°C w/ EDTA, maybe 10% loss of activity.

$$140 \text{ pmole} \left(\frac{23.1}{2} \right) / 10,000 \times 3 = 0.49 \mu\text{l} \text{ (expected 0.5)}$$

A no killing
A 90° 5'
A 90° 10'
A 90° 30'
A 90° 1hr
B no killing
B 90° 5'
B 90° 10'
B 90° 30'
B 90° 1hr
A 75° 30'
A 75° 1hr
A 75° 2hr
A 75° 4hr
B 75° 30'
B 75° 1hr
B 75° 2hr
B 75° 4hr
Tne, no heat or EDTA
Tne, 90° 10'
Tne, 90° 1hr
Tne, 75° 1hr
Tne, 75° 4hr

A = 50 units Tne : 5 units DNase I
B = 50 units Tne : 1 unit DNase I

Nicking assay - after treat to kill DNase I, the rxn incubated w/ 1 μg of X174 for 3 hr at 23°C. to rxn any remaining DNase I supercoiled

note that no untreated DNA was run as a pool control for how 1 μg looks. the Tne lanes serve as a control because Lig^{III} show Tne has no endonuclease. The 75°C 4hr treatment EDTA and the lower level DNase was best for kill the low level of DNase I degrades 1 μg easily

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21/95

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8/17/95

T Page 1

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treatment of Tne with DNase I: 2 rxns of

87.6 μ l H₂O

10 μ l 10x DNase I buffer ~~CF=0.5~~

1.39 μ l Tne, 36 μ l, 5-7-95 prep

1 μ l DNase I, 1 μ l

100 μ l

test in PCR
unfilled

remove 10 μ l to 400

90 μ l 1 rxn 30 min RT

1 rxn 4 hr RT
+ 15 min

+ 5.74 μ l 50mM EDTA CF = 3mM

new vol = 95.74 μ l CF_{Tne} = 0.47 μ l

at 75°C for 4 hr in 9600 PCR machine
12 AM - 4 PM, put on ice

control reactions without DNase I: 2 rxns of

88.6 μ l H₂O

10 μ l 10x DNase I buffer

1.39 μ l Tne, 36 μ l, 5-7-95

100 μ l

mock reaction

remove 10 μ l from each to test in PCR

i.e. no killing treatment

90 μ l 1 rxn 30 min RT

1 rxn 4 hr RT
+ 15 min

+ 5.74 μ l 50mM EDTA CF = 0.47 μ l
Tne

at 75°C for 4 hr in 9600
put on ice

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Paulson Conf

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8/17/95

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PCR rxns: 0.5, 1, 2, 4 units Tne
 done 8/17/95
 long smear conditions and conditions to make specific
 - test DNase I treated Tne
 - test Tne that has been through a mock DNase I treatr.
 - test fresh Tne

mix A - to make a long smear

- for 14 rxns 1151.22ul H₂O

28ul 1M Tris 8.5 C_f = 20mM

note: in the rxn with
 4 units Tne, the
 [KCl] will be 55mM

23.38ul 3M KCl C_f = 50mM

29.4ul 50mM MgCl₂ C_f = 1.05mM

28ul 10mM dNTPs C_f = 200uM

1260ul

mix B - to make 380bp product

- for 14 rxns 1043.98ul H₂O

28ul 1M Tris 8.5

~~39.42ul~~ 39.42ul 3M KCl C_f = 85mM

30.4ul 50mM MgCl₂ C_f = 1.3mM

28ul 10mM dNTPs C_f = 200uM

dilution of fresh Tne

30.4ul 5-7-95 stock

2ul stock

151.2ul Tne 513

153.2ul of 0.4pu/ul

28ul 20uM anchor primer C_f = 400nM

28ul 20uM 6681 primer C_f = 400nM

28ul 50pg/ul M13 RF C_f = 100pg/rxn

mix A 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24
 90 ————— 1

mix B 90 ————— 1
 H₂O 8.9 7.87 5.74 1.49 8.9 7.87 5.74 1.49 8.9 7.87 5.74 1.49 8.9 7.87 5.74 1.49 8.9 7.87 5.74 1.49

DNase treated Tne 1.1 2.13 4.26 8.51 1.1 2.13 4.26 8.51

mock treated Tne 1.1 2.13 4.26 8.51 1.1 2.13 4.26 8.51

*fresh Tne 1.1 2.13 4.26 8.51 1.1 2.13 4.26 8.51

100ul rxns started on ice.

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R corded by

Paula Smith

8/18/95

g N .

R in Lab 15 9600 - 1 min 94°C

35
4105 (30 sec 94°C
30 sec 55°C annealing
2 min 72°C elongation
4°C

program 76
method links 71, 75, 74

50 mM KCl
1.05 mM MgCl₂

85 mM KCl
1.3 mM MgCl₂

The treated w/ DNase I
for 30' so the
1.0 DNase I

7-95 enzyme
units

Tne w/ DNase				mock				fresh				Tne w/ DNase				mock				fresh			
0.5	1	2	4	0.5	1	2	4	0.5	1	2	4	0.5	1	2	4	0.5	1	2	4	0.5	1	2	4

Mock rxn = Tne. without
DNase I taken
through all the
DNase I treatment
steps

Fresh = untreated
The used directly
from -20°C stock



8/18/95
cc

ste: 85 mM KCl did not prevent the small smear from forming

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follow P 138, 9

lot CK041

lot EJP41

no
enzyme

①

②

③

2 x 1

Rmix (B&D system)

147.5 μ l147.5 μ l19.67 μ l

= 0.1

20

0.2

1750

7

* see P. 75

Klenow exo(-)

lot

CK041 P63, 7541
dil in Tag SB to 4 μ l

2.5

20

EJP41 P63, 13041
dil in Tag SB to 4 μ l150 μ l

2.5

150 μ l0.33 μ l Tag SB20 μ l2.2 μ l BJ

41°C in 9:00

remove 20 μ l to 2.2 μ l 10xBJ + 100 mM EDTA
at 3 min, 1, 2, 5, 30, 90, 2 hr

start 1:2

well#

2-7

8-13

load on 8% Agarose

③

①

②

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T Pag

Experiments on DNase I treated Tne
Mg²⁺ titration & mixing expt.

g N _____

crase. To determine if & how Tne was damaged by the DNase I treatment. p. 178 back rxns show low polymerase activity in the PCR, so the 75°C treatment w/ EDTA affected Tne even if DNase I was not present. A unit assay showed most of the activity was still present (~25% died). Today, we'll add back Mg²⁺ in case there was more free EDTA than we thought. We'll also try poisoning a fresh Tne rxn w/ the treated Tne.

Mg²⁺ titration 1.05, 1.3, 2 mM MgOAc
smear & product conditions
1 unit DNase treated Tne (30' treatment)
~~1 unit fresh Tne~~
1 unit fresh Tne from 5^u/ul stock

ar mix for 12 rxn → 24ul 1M Tricine pH 9 Cf = 20mM
30ul 2M KOAc Cf = 50mM
24ul 10mM dNTPs Cf = 200uM
88.2ul H₂O
960ul

↓ [B]
280ul mix
14.7ul 25mM MgOAc
Cf = 1.05mM
20.3ul H₂O
use 90ul/rxn

↓ [C]
280ul mix
18.2ul 25mM MgOAc
16.8ul H₂O
Cf = 1.3mM

↓ [D]
280ul mix
+ 28ul 25mM MgOAc
7ul H₂O
Cf = 2mM

ul mix^B + 3 ul Tne treated w/ DNase 30' + 7ul H₂O = 100ul * Tne at 0.33^u/ul, as determined by unit assay p. 73 NB 10 & p. 180 NB 11
1 mix^B + 3ul Fresh Tne^Δ + 7ul H₂O Δ diluted 5^u/ul stock to 0.33^u/ul
5ul of 5^u/ul Tne 5-7-95
70.8ul of Taq SB
75.8ul of 0.33^u/ul Fresh Tne
ul mix C + 3ul treated Tne + 7ul H₂O
ul mix C + 3ul Fresh Tne + 7ul H₂O
ul mix D + 3ul treated Tne + 7ul H₂O
ul mix D + 3ul Fresh Tne + 7ul H₂O

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SAP CPMI

1	2952.00
2	2739.00
3	4871.00
4	3459.00

2846

4115

68%

} The (ONase treated 30') dil $\frac{1}{23.5}$ } Lig. The stock 5% 5-7-85
diluted to 0.4% (same as ONase)
treated above and then diluted
 $\frac{1}{23.5}$ unit assay ^{same} as P73, 10

conclude The lost ~70% activity from
killing 4 hr killing of ONase I at 75°C (P175)

product mix for Mg^{2+} titration:for 12, 100ul rxns = 24ul 1M Tricine pH 9 $C_f = 20mM$ 51ul 2M KOAc $C_f = 85mM$ 24ul 10mM dNTPs $C_f = 200uM$ 24ul 20uM 6681 primer $C_f = 400nM$ 24ul 20uM anchor primer $C_f = 400nM$ 24ul M13RF, 50pg/ul $C_f = 100pg/rxn$ 789ul H_2O

960ul

Ⓔ

for 3.5 rxns

280ul mix

14.7ul 25mM $MgCl_2$ ←
20.3ul H_2O $C_f = 1.05mM$

315ul

use 90ul/rxn

Ⓕ

280ul mix

+ 18.2ul 25mM $MgCl_2$ ←
16.8ul H_2O

315ul

 $C_f = 1.3mM$

Ⓖ

280ul mix

+ 28ul 25mM $MgCl_2$ ←
+ 7ul H_2O

315ul

 $C_f = 2mM$

J. Polak

8/28/95

D. L. L. L.

8/22/95

ag N _____

reactions → DT = Tne. treated w/ DNase for 30' p. 175, 1 unit, 0.33%
 FT = Fresh untreated Tne diluted to 0.33%^{vol}, 1 unit

smear condition
 50 mM KOAc
 20 mM Tricine pH 9
 no template no primers

product condition =
 85 mM KOAc
 20 mM Tricine pH 9
 anchor primer + 6681 primer on m13

[KOAc] mM	1.05		1.3		2 mM		1.05		1.3		2	
zyme	DT	FT	DT	FT	DT	FT	DT	FT	DT	FT	DT	FT
1 unit of eng./rxn												
0.8	1	3	4	6	7	9	10	12	13	15	16	18
20ul of each 100ul rxn was run on a 0.8% gel p. 184 →												

We had also planned to do rxns with 4 units of the DNase I treated Tne, but there was not enough of the eng. to set up these rxns

~~Mg²⁺ was omitted from rxn by mistake~~
 being experiment to determine if the DNase I-treated Tne has
 a "poisonous" substance in it - mix untreated Tne w/ DNase-
 treated Tne. smear & product conditions 1 u untreated + 0 treated

mix for smear, 6 rxns = 12ul 1M Tricine pH 9 (C=20mM)
 15ul 2M KOAc C=50mM
 12ul 10mM dNTPs C=200uM
 441ul H₂O

480ul → use 80ul/100ul rxn

80ul mix + 3ul fresh Tne (0.33%^{vol}) + 17ul H₂O
 80ul mix + 3ul " + 1.52ul treated Tne + 15.48ul H₂O
 80ul mix + 3ul " + 3ul " + 14ul H₂O
 80ul mix + 3ul " + 6ul " + 11ul H₂O
 80ul mix + 3ul " + 12ul " + 5ul H₂O

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Paula Lynn

Date

8/21/95

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2nd try at the mixing expt. - I forgot to add MgOAc to the rxns on

smear buffer: C = 20mM Tricine pH 9.0
50mM KOAc
1.05mM MgOAc
200μM dNTPs

no primers or template
mix 1 unit of fresh Tne w/
0, 0.5, 1, 2, 4 units of
mock-treated Tne. p. 17:

for 6 rxns = 12ul 1M Tricine pH 9 ✓

15ul 2M KOAc ✓

25.2ul 25mM MgOAc ✓

12ul 10mM dNTPs ✓

41.5.8ul H₂O ✓

480ul → use 80ul mix per 1, 100ul rxn

product buffer: C = 20mM Tricine pH 9

85mM KOAc

1.3mM MgOAc

200μM dNTPs

400nM 6681 primer

400nM anchor primer

100 pg/1rxn M13mp19 RF template

mix 1 unit fresh Tne w/
0, 0.5, 1, 2, 4 unit
of mock treated Tne
p. 17

A unit assay was done on the ^{mock} treated Tne on Fri 8/18/95 p. 75 Nk
We'll assume a concentration of 0.33^u/ul, which was the an
concentration on 8/18. The base treated Tne had not lost any more acti
at 4°C over 3 days, as shown by unit assay on 8/21/95 p. 180. It's likely
the mock didn't loose an

for 6 rxns = 12ul 1M Tricine pH 9 ✓

25.5ul 2M KOAc ✓

31.2ul 25mM MgOAc ✓

12ul 10mM dNTPs ✓

12ul 20μM 6681 ✓

12ul 20μM anchor ✓

12ul 50 pg/ul M13 RF in TE ✓

363.3ul H₂O ✓

480ul, use 80ul mix / 100ul rxn

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Date

8/22/95

Tag N	19	20	21	22	23	24	25	26	27	28	29
1st mix	80ul										
2nd mix						80ul					
Tne	3ul (unit)										
2nd-treated	0	1.52	3	6	12	0	1.52	3	6	12	
0.33% w/v											
5											
17	15.48	14	11	5		17	15.48	14	11	5	
100ul											

94°C 1 min

94°C 30 sec

55°C 30 sec

72°C 2 min

4°C - hold

Method 76, Lab 15 9600 Method 103 Lab 16 9600

35 cycles

Dilution of fresh Tne : 5ul of 5% Tne 5-7-95 Lys stock
70.8ul Tne SB

75.8ul of 0.33% w/v fresh Tne

3% TAE gel w/ cTBr, 20ul of each rxn was run on gel

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Results:

Mg^{2+} titration
 p. 179
 DT = Tne treated 30' with
 DNase I p. 175
 FT = fresh, untreated Tne
 smear cond. ✓ product cond.
 50 mM KOAC 85 mM KOAC
 no template/primer

Mixing expt.

p. 182
1 unit Fresh Tne+ increasing units
of mock treated

Tne (no DNase)

smear cond. product cond.
units of mock treated

Rnase treatment of Tne

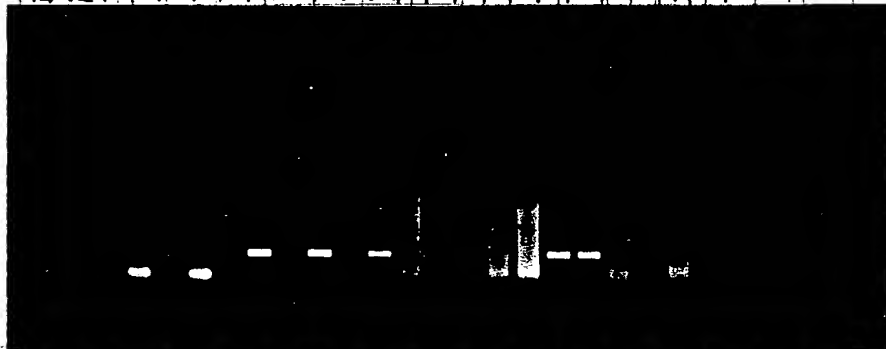
p. 185

+ = Tne treated w/ RNase

- = Tne not treated w/ RNase

1 unit eng →

1.05 1.3 2 1.05 1.3 2
 0.5 1 2 4 0.5 1 2 4 + - + -

CSE
8/23/95Conclusions from Mg^{2+} titration expt:

A unit assay was done on the DNase I treated Tne (see p. 180) and fresh Tne. Equal units of the DNase I treated and fresh Tne were used in the Mg^{2+} titration PCR reactions. The treated Tne did not make any specific product, while the Fresh Tne did. We conclude that the 75°C incubation with EDTA (to kill the DNase I) damaged Tne. Therefore we do not yet know if treating Tne w/ DNase I can eliminate the "bad seed" DNA and prevent a smear.

If there was some residual DNase I activity, the product & smear could be degraded. 2 pieces of data argue against the active DNase explanation. 1) our nicking assay p. 174 shows that 1 µg of p174 was not degraded in 3 hr and was only nicked a little (~10%) at the DNase I killing treatment. 2) in a previous expt (p. 177) the mock-treated Tne showed the same low activity in a PCR as the DNase I treated Tne. So, the DNase was not responsible for the low amount of smear products made.

Tne + EDTA died at 94°C. maybe adding more EDTA can overcome the damage to Tne. p. 174

Further expts to try: measure thermostability of the 75°C w/ EDTA treated Tne, by cycling the Tne to 94°C before doing the unit assay → purify the DNase I-treated Tne away from DNase or "poison"-gel

Witnessed & Understood by me,

Date

Inventor by

Date

S. O. O. O. O.

8/28/95

Recorded by

Dariusz Pami

8/23/95

To Page No.

Expt.: RNase A + 1 + treatment of
Tne. Is RNA the bad seed that
primes the smear.

Pr j ct No. _____

185

PCR 1 is 1+2

PCR 3 is 5+6

See result
on p. 184

PCR 2 is 3+4

PCR 4 is 7+7

apt 3 → RNase

(#1, ~~2~~)

small worth of buffer w/ 100ul worth of dNTP, target, primers

smear V V 1ul Tricine

V V 1.25ul 2m KOAc

V V 2.1 ul 25mM MgOAc

V V 2 ul 10mM dNTP

V V 2ul 6681 20mM

V V 2ul anchor

V V 2ul m13, 50pgul

1 RNase T1 } both diluted 10 fold
1 RNase A } in 10 mM Tricine

35.65 ul H₂O

50ul

T1 1460u/ul

A 10mg/mL = 10ug/ul

1 ul → 40ug RNA/mL

dil 10x
use
1ul

car Tne V V

1ul Tricine (#2)

V V

1.25ul 2m KOAc

V V

2.1ul 25mM MgOAc

int
total →

2ul Tne

Li25uA

want 1 unit
→ dil to 0.5uA in SB

1 ul RNase I

1 ul RNase A } dil 10x in
10mM Tricine

ul H₂O

V V

46.65ul

50

15' 37°C → mix → PCR

same w/o RNase

(#3, 4)

ESC
8/22/95

Read & Understood by me,

J Polansky

Date

8/28/95

Invented by

Recorded by

Paula Combs

Date

8/22/95

From Page No. _____

expt 3 RNase:

for product

1ul Tricine 1M

✓ ✓ 2.13ul 2M KOAc

✓ ✓ 2.6 ul 25mM MgOAc

✓ ✓ 2ul dNTP 10mM

✓ ✓ 2ul 6681 20mM

✓ ✓ 2ul anchor 20mM

✓ ✓ 2ul m13 50pg/ul

1 RNase

1 RNase

✓ ✓ 34.27 H₂O

50ul

✓ ✓ 1 ul Tricine

✓ ✓ 2.13 2M KOAc

✓ ✓ 2.6 25mM MgOAc

2 ul Tric 0.5u/l

✓ ✓ mix 40.27 H₂O

1 RNase

~~it~~✓ ⁵⁰ same w/o RNase

#5

8/22/95
CS

#6

#7

#8

T Pag 1

With ss d & Und rstood by me,

Dat

8/28/95

Inv nt d by

Record d by

Paula Pauli

Dat

8/22/95

g N 184

conclusions from the mixing expt. on p. 184:

The purpose of the mixing expt was to see if 0.5, 1, 2, 4 units of mock treated Tne could poison a PCR with 1 unit of fresh Tne. The mock treated Tne received the 4hr 75°C EDTA treatment but did not contain any DNase I.

The mock-treated Tne did not poison the ability of fresh Tne to make a ^{long} smear under the standard smear buffer conditions of 50mM KClAc, 20mM Tricine, 4.05mM MgOAc. The differences in smear intensity are probably just representative of ^{typical} variation in smear intensity. Therefore the mock-treated Tne does not contain a poison that is effective at the levels tested.

The mock-treated Tne also did not poison fresh Tne's ability to make a specific product (0.5u mock w/ 1u fresh still made product). The smears seen with 1, 2, 4u mock probably are the result from having too many total units. Using more than 1 unit Tne/100ul rxn always results in a smear. The amt of mock was not enough to make a ~~smear~~ 1 unit mock may not be exactly the same as 1 unit of treated (which doesn't show any PCR activity in the Mg²⁺ titration expt) and the window for ~~too~~ activity is probably very narrow. - More controls & expt should be done to confirm the absence of a poison.

conclusions from RNase expt - Tne treated with RNase A + RNase T₁ and then used directly in a PCR. If RNA is the "bad seed" RNase might cure the formation of a smear and increase product yield. The RNase treatment had no effect on either smear formation or product yield. We conclude that RNA is not priming the smear reaction.

To Page No. _____

ed & Understood by me,

Polansky

Date

8/28/95

Invented by

Recorded by

C. E. F. F. F.

Date

8/22/95

TITLE

From Page No.____

Results:

DT = Tine treated 30' with
Nase I n.175

FT = fresh, untreated Tne smear cond.	✓ product cond.
50mm KOAc	85mm KOAc
no template/primer	

Mixing expt.
p. 183

Unit Fresh Tne

+ increasing units
of mock treated

Tr4 (no DNase)

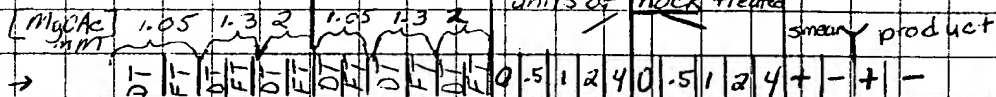
Rnase treatment of T₁e

p. 185

f = Tne treated w/ RNase

→ = Tne not treated w/ RNase

1 unit enz \rightarrow



CSL
8/23/45

Conclusions from Mg^{2+} titration expt:

A unit assay was done on the DNase I treated Tne (see p 180) and fresh Tne. Equal units of the DNase I treated and fresh Tne were used in the Mg^{2+} titration PCR reactions. The treated Tne did not make any specific product, while the Fresh Tne did. We conclude that the $75^{\circ}C$ incubation with EDTA (to kill the DNase I) damage Tne. Therefore we do not yet know if treating Tne w/ DNase can eliminate the "bad seed" DNA and prevent a smear.

If there was some residual DNase I activity, the product & smear could be degraded. 2 pieces of data argue against the active DNase explanation. 1) our nicking assay p. 174 shows that 1 μ g of ϕ X174 was not degraded in 3 hr and was only nicked a little (~10%) after the DNase I killing treatment. 2) in a previous expt (p. 177) the mock-treated Tne showed the same low activity in a PCR as the DNase I treated Tne. So, the DNase was not responsible for the low amount of smear products made.

Time + EDTA dies maybe
at 9:45 adding more back
comp. are become the
duminate to time
p. 174

Further expts to try: → measure thermostability of the 75°C w/ EDTA treated Tne, by cycling the Tne to 94°C before doing the unit assay
→ purify the DNase I-treated Tne away from DNase or "poison"-gel

Witnessed & Underst d by m ,

Dat

Inv nt d by

Dat

Recorded by

8123/95

ag N _____

2.2 X reactions, $V_p = 0.1$ each

Rx mix (B&D)

Cf at 1X

2. HPO₄ pH 7.6
P61

~~33~~ μ l

16.5 μ l ✓

50 mM

2 NTP₂ 10 mM

~~130.2~~ μ l

6.6 ✓

200 μ M each

BSA nuclear free

~~4.4~~ μ l

2.2 ✓

0.1 mg/ μ l

chelated Strategic cat 80004157

~~180.84~~ μ l

~~90.4~~ 86.9 ✓

13.17 % (includes contributed by Klenow ex.)

50 % glycerol

~~4.60~~

2.31 ✓

7 mM

1 M Mg Cl₂

2 must go in after

204 is diluted to

1. ppt of Mg PO₄)

33 correct. mp 19

~~52.8~~ μ l

26.4 ✓

138.9: 41/50 μ l rxn vol

0.12 pmol primer

0.24 pmol circle

circle primer = 2

note 5 μ l K_{Cl}

295 μ l rxn

contributes

$(\frac{5}{300})$ 50 % glycerol

= 0.833 %

to Cf

∴ overall Cf

is 140 % glycerol

in Rxn

H₂O

~~360.14~~

183.6 ✓

~~180.1~~

$V_p = \frac{649}{324.5}$

To Page No. _____

Issued & Understood by me,

J. B. Jones

Date

8/28/95

Invented by

F. C. C.

Date

7-25-95

Recorded by

From Page No. _____

mix A

for 4 Rxns (Cf 100)

10X PCR buffer
50 mM MgCl₂✓ 40
✓ 56(Cf = 1100 CPM/pmol &
G 200 μ M each)H₂O✓ 260
356use 95 μ l / 100 μ l Rxn

(1) (2) (3)

m A

89 μ l

→

✓ (53.6 u)

Tne. Ciz 36 μ l

2.6

✓

Tne P L 7-22-95

19 μ l (acc P 127, 11)

5

✓

95 u

rTos EKBTI

dil to 36 μ l

2.6

✓

93.6

Tng SB

2.4

2.4

✓

heat reactions 1' 94
then lower heat to 7
then start reaction

dCTP, dATP, dTTP

6
✓ 100 μ lby addition
of dCTP, dATP
ring

X mix

3' P dCTP 16
100 μ lremove 10 μ l to 5 μ l cycle stop solution
at 1 2 5 15 30 60 90 min10 min dATP 8
✓ = 64

run of 2% PAGE

with dDA, dAT and PFI (Reactions #22, 23, 24
at top of P7P) reloaded here as
number 22, 23, 24 also. 23 is no Eng

T Page 8

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S. Polans

Dat

8/28/95

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Record d by

Dat

8-28-95

86

see P155-157 within

Project No. _____

Book No. _____

TITLE

Provisionality

The FY

vs 1

vs Althman

From Page No. 60811

Mix A process

(for 37 Rxns, 40 μ l/5 μ l R

from P.75

32 P33 run correct - mp19

✓ ✓

130 μ l

7.8 pmol circles

circle/prime = 2

3.9 pmol prime

0.06 pmol circle

H₂O

✓ ✓

1109.5 μ l

for 37 Rxns

10 x PCR buffer

✓ ✓

147 (1x at 40 μ l)use 40 μ l/Rxn50 mM MgCl₂

✓ ✓

55.5 μ l (1x at 50 μ l)

Cp = 1.5 mM Mg

10 mM DTPS

✓ ✓

37 (1x at 50 μ l)1x at 50 μ l Rxn = 21

μ l = 1.47 μ l
 3.84 x The Liz well H
 The FY
 5.4 μ l
 (Altman
 5.4 x)

sol dil	.00005 μ l
	.0001
	.0002
	.0004
	.0008
	.0016
	.0032
	.0064
	.0128
	.0256
	.0512

#1

12

23

2

13

24

3

14

25

4

15

26

5

16

27

6

17

28

7

18

29

8

19

30

9

20

31

10

21

32

11

22

33

for 40 μ l mix

= 0.21 pmol

50 μ l
n same as

put 2 μ l pol into 8 μ l of 1.25 x PCR buffer
 preheat to 70°C 1 min in 9600

start with 40 μ l of Mix A process (also preheated to 70
 stop at 2 min with 25 μ l cycle seq stop sol

number "0" is Mix A process 40 μ l
 Tag SB 2
 H₂O 8
 cycle seq stop 50 μ l
 25 μ l

To Page 1

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Dat

Invent d by

Dat

a/7/95

R c rded by

J-8795

From Page No. —

Fidel Pri (P54)
100 μ M
(its a 27mer)5 μ l ✓

(500 pmol total)

 γ -³²P ATP 10 mCi/ml
(3.33 μ M)

10 ✓

(33 pmol total)

5X Knaal buffer
PNK 10⁴ U
H₂O

10 ✓

24 ✓

50 μ l

37°C, 30' → 70°C, 5'

[dT]

25
5

[dA]

25
5

✓

Fidel Temp (dT)

Fidel Temp dA

P54 100 μ M

10 mM Tris pH 8

247.78

277.8

5 μ l ✓5 μ l

500

(1000 pmol)
($\frac{500 \text{ Temp}}{25 \text{ pri}} = 2$)

Cf = 900 nM primer

↓

90°C 2 min

↓

cool slow

use 5 μ l / 50 μ l extension reaction
for Cf = 90 nM primer

Witness d & Understood by me,

Date

9/7/95

Invent d by

Rec rd d by

Dat

8-31-95

T Pag Nc

From Page No. _____

(14 Rxns)

I

II

14 Rxns

200 μ l of 2.0 μ l each of 5x

5x Cheung (+JNTPs)

70 μ l

✓

10.05 mM Mg

10x PCR buffer

70

✓

50 mM MgCl₂

14.7

✓

1.05 mM Mg

10 mM JNTPs

14

✓

Human DNA spleen

7 14

7 14

✓

10 μ M $\frac{1}{2}$ 267 bp $\frac{1}{2}$

14 28

14 28

✓

H₂O

534 532

552.3 28
545.3 503.3

✓

400 μ l

inc 47 μ l/Rxn

672

672

I

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25

II

48 → 45

The wt (Lys 1-755)

544

Temp SB

0.25 μ l

2

6

6

2

0.4
2.5 μ l of
544/1.1

0.5

2

6

6

2

1

2

2.5

10

2

1.5

2

2

4.67

2

2

2

2

3

✓

The Δ 5 FY 5.4 μ l SB

0.25

5.5

5.5

2

2

0.5

5.5

5.5

2

2

1

1.2

10.08

2

2

1.5

1

5.27

2

2

2

1.5

5.55

2

2

3

2

4.27

2

2

4

2.5

3.37

2

2

50 μ l

T Pag N

With ss d & Underst d by me,

Dat

Inv nt d by

Dat

9/7/95

9/7/95

R c rd d by

9-1-95

PCR with OAPDH, gloom, Chery vs PCR buffer
 for The WT and ΔSFY
 see P 78, 11 for wt conditions

Proj # No. _____

B k No. _____

93

Tag No. _____

94°C 1 min

94°C 30 s

55°C 30 s

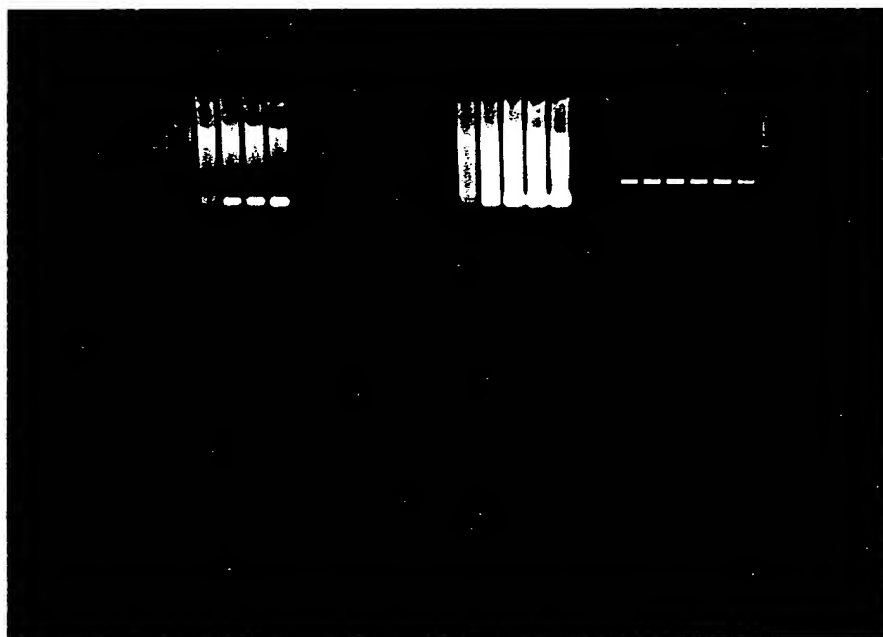
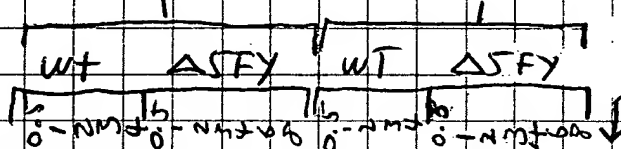
72°C 2 min

35 cycles

4°C hold

(set BS+EDTA added ≤ 30' after finish
 Chery buffer Tag PCR buffer
 2 units
 r Tag pol
 in Tag PCR buffer

The
 units



← 267

Result: only get expected 267 bp product for The ΔSFY if
 PCR buffer used consistent with lower ionic strength
 of PCR buffer helping the less processive deleted pol
 (Chery is 55 mM KOAC compared to 10 mM)

To Page No. _____

Used & Understood by me,

Date

Invented by

Dat

S. Polansky

9/2/95

Recorded by

9-1-95

PCR with Tne Δ5 FY

Project

Book No.

TITLE

267bp - 7.5 bp products

94

From Page No.

Mix A

10x PCR buffer
 5x Thermo + dNTPs
 50 mM MgCl₂
 Hammer DNA 20 ng/μl
 H₂O
 Tne Δ5 FY

100 ✓ for 20 PCR
 200 μl ✓
 21 μl ✓
 20 μl ✓
 810.5 25.5 ✓
 8.5 μl ✓
 980 μl ✓

	1	2	3	4	5	6	7	8	9
mix A	48 μl								
267bp 16/12 h/g									
BDNF 10 μM primers		1							
1.366 Kb primers			1						
2.0				1					
2.82					1				
4.1						1			
5.5							1		
6.166								1	
7.5									1

94°C, 1'

94°C 30S
 15°C 30S } 35 cycles
 72°C

40°C

2 min (#1-9) or 7 min (#10-18)
 (start 11:35) (start 11:25)
 (get BJ + EDTA in ≤ 30' after finish
 in case 3' ext is a problem)

start 7:40
 for 7 min along need ~ 5 hrs
 done ~ 2:30
 needs 2 1/2 hr for 2 min e
 (done ~ 11:10)

Witnessed & Understood by me,

SO Ocamp

Dat

9/2/95

Invent d by

R c rded by

Dat

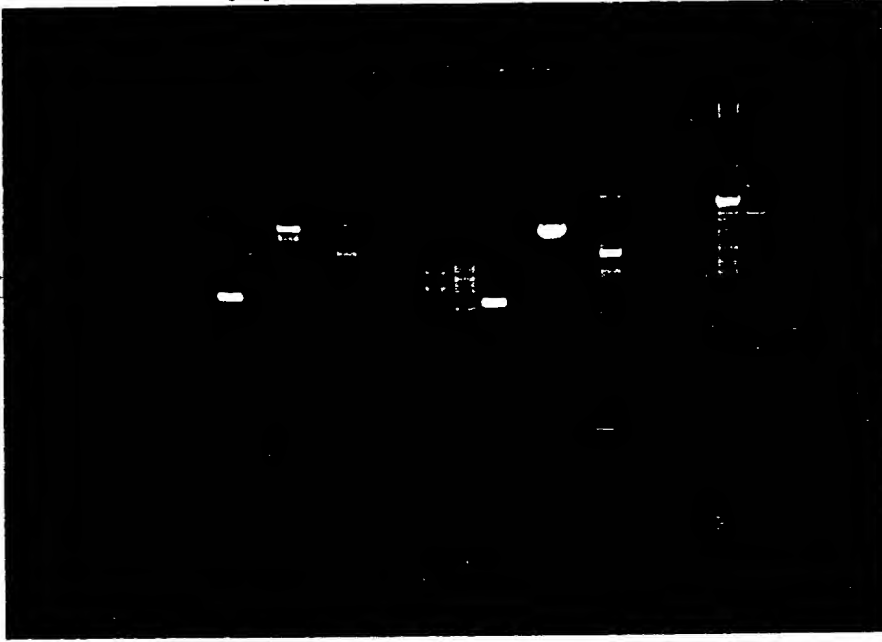
9-6-95

To Pag No

Page N _____

2' elongation 7' elongation

267 200 200 4.1 5.5 6.1 7.1



267
200
1.36
2
2.8
4.1
5.5
6.1
7.1

+

+

+

+

-

-

-

-

-

(for 7 min elongation

(for 7 min

To Page No. _____

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J. Polansky

Date

9/7/95

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Date

9-8-95

From Page No. _____

10x T_{aq} PCR buffer = 200 mM Tris pH 8.4, 500 mM KCl

10x ultimate buffer is 100 mM Tris pH 8.8, 10 mM KCl

I will try 0 10 25 50 mM KCl CF
 (for now I'll stay with 20 mM CF Tris 8.8)

(A)

1 M Tris pH 8.8

20 μ l

✓ (CF=20 mM)

(for 10x 10)

50 mM MgCl₂21 μ l

✓ (CF=1.05 mM)

4 dNTPs

20

✓ (200 μ M each)

Human DNA 20 ng/1

5 μ l9.4 μ l T_{aq} D5FY8.5 μ l

✓

(4 μ l/50 μ l as per PH₂O86.5 μ l

✓

24/100 μ l PCR

940

use 94 μ l/100 μ l F

(A)

① ② ③ ④ ⑤ ⑥ ⑦ ⑧
 94 μ l

KCl

—

—

✓

200 mM

✓

✓

(CF= 1)

500 mM

✓

✓

2

1 M

✓

✓

3

primers

267 bp 16/12 h/g 1 —————→

1.366 kb

1 —————→

H₂O

5
 100 μ l

cycle as per P 94 with 2 min elongation

start 7:40 AM

done

T Page

Witnessed & Understood by me,

S. Olamp

Date

9/7/95

Invested by

Recorded by

Date

9-7-95

e N . _____

Result

note 267 bp product does
just begin to appear
at 5 cycles and
highest No. 4

cell of 10 mM
concluded:

- 1) higher NaCl
help in contrast
to expectation that
it would inhibit
this distributive
form of Tse.
2) target DNA
is limiting here
since higher yields
were obtained for
4x more DNA in
P 93 and 94

apparently need $\geq 1 \mu\text{g}$ of H/S DNA (100 ng) / 50 μl Rxn
need only 0.25 μl in

To Pag No. _____

sed & Understood by me,

Date

Invented by

Date

R corded by

Sandra B. King

9/7/95

9-7-95

Prepare Tth sol for shipping
to Roache

98

Book No. _____ TITLE _____

From Page No.____

They want 10,000 units and some SB as LTI except include 100 mm K&L

Therefore

Tth (formerly thought to be Tfi)
4-30-95 (see P for units)
4.33 u/μl

2.5 ml

(10825 units
total)

*. 2 M KCl in 50% glycerol
and 2.0 mM Tris pH 8

0.132 ml

$$V_f = 2.632 \text{ mL}$$

final units = $14.1 \text{ units}/\mu\text{L}$

* Kel
glycerol
H₂O
in Tris pH 8

O. 298

8

$$(mw = 74.5)$$

1 ml

To 2 ml ✓

20 ml

$V_f = 2 \text{ ml}$

To Page No

Witnessed & Understood by me,

Date

Invented by,

Date

Research Policy

9 | 9 | 95

Recorded by

7-950